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THE ANATOMY OF THE SACRO-UTERINE LIGAMENTS

FRANK E. BLAISDELL, SR.

*From the Laboratory of Surgical Pathology of the Medical School of Stanford
University*

TWENTY-TWO FIGURES

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I. INTRODUCTORY

A great deal has been written upon the anatomy of the pelvic floor and the mechanical supports of the pelvic viscera. While all of the contributors have more or less advanced our knowledge of the anatomy of the pelvis, all have failed to completely elucidate the anatomical and physiological importance of the fibro-elastic tissue that forms a more or less dense mesh-work, filling up the interval between the peritoneum above; the pelvic diaphragm covered by its fascial sheath below; the obturator fascia and periosteum of the innominate bone laterally; the bladder, vagina, and uterus with their fibrous investment medially; and finally, the fascia over the sacral promontory, sheath of the sacral plexus, and fibrous tunic of the rectum, posteriorly. Cameron and Moritz have made the nearest approach to the proper solution of the question, but they failed in not subjecting the 'compact mass' filling up the interval between the peritoneum and pelvic floor as above limited to the proper anatomical analysis.

The primary object of the present investigation is the study of the so-called sacro-uterine ligaments; to determine what they really are and to establish their relation to the transverse ligaments of the uterine neck, recto-uterine muscles and recto-uterine folds.

II. METHODS OF STUDY AND MATERIAL

From a survey of the work which has been done, it is evident that a comparative and a more comprehensive study is absolutely necessary for the proper solution of the problem. In accordance with that view, cadavers that had been embalmed in the usual manner for dissection, and others preserved with 10 to 40 per cent solutions of formaldehyde, and fresh material in and from the autopsy room, were used. Recent or unfixed material has the advantage of being perfectly flexible, permitting of experimental procedures not possible on the firm and inelastic material of the dissecting room. Finally, a careful study of frozen sections revealed some most interesting points.

The human material included fetuses, the new born, and cadavers of the following ages: 40 days, 10, 24, 35, 65, and 83 years. The animals most readily available were the guinea pig (*Cavia cabayia*), Belgian hare (*Lepus europaeus*), cat (*Felix domestica*), dog (*Canis familiaris*), and monkey (*Macacus*). In the whole series the parametrium containing the so-called ligaments and folds were cut in serial sections and subjected to a careful examination. Experimental observations on the pelvic organs were made on the animals while under an anesthetic.

Since the appearance of a preliminary report of this paper in June, 1913, there has been published an interesting paper by Moritz ('14), in which the author considers the distribution and significance of the parametrium. His method has been to obtain the pelvic contents as soon after death as possible, by having them removed right down to the bone and the pelvic floor cut away with them. The specimens were then fixed in 10 per cent solution of commercial formalin. Not 'dissected,' in the ordinary acceptation of the term, but sections were cut in varying planes and directions.

III. BRIEF REVIEW OF THE LITERATURE

a. Chronological

The more important contributors are the following:

Kocks, (1) ('80),	Derry, (10) ('07),
Hart, (2) ('80),	Paterson, (11) ('07),
Mackenrodt, (3) ('95),	Ovenden, (12) ('07),
Holl, (4) ('97),	Cameron, (13) ('07-'08),
Harman, (5) ('98),	Smith, (14) ('08),
Thompson, (6) ('00-'06),	Fothergill, (15) ('08),
Deaver, (7) ('03),	Somers and Blaisdell, (16) ('13),
Montgomery, (8) ('03),	Moritz, (17) ('14).
Stony, (9) ('04),	

Kocks (1) described the cardinal ligaments. Mackenrodt (3) defined the ligamentum transversale colli. Deaver (7) states that the false posterior or recto-uterine ligaments are composed of two peritoneal layers which pass backward from the posterior surface of the uterus and vagina to the upper portion of the rectum, forming the lateral boundaries of the pouch of Douglas; external to each are found the true or muscular utero-sacral ligaments, which are flat muscular bands that extend from the uterus at the level of the internal os, to the sides of the sacrum, passing beneath the layers of the recto-uterine ligaments.

Montgomery (8) says that the utero-sacral ligaments, while consisting of folds of peritoneum, also contain muscle fibers, which are derived from the superior muscular layer of the uterus.

Paterson (11) considered the suspensory ligaments. Ovenden (12) reviews Mackenrodt's work and states that the ligamentum transversale colli is worthy of being recognized as a distinct ligament, and that the utero-sacral ligament blends with the former near its insertion into the uterus.

Cameron (13) considers the utero-sacral ligaments as a part of the general perivascular mass.

Moritz (17) in criticizing Dr. Ovenden's views states: "I hope to demonstrate that the sketch she published of the insertion of the ligament (transverse of the neck) shows clearly that the so-called ligament is simply a portion of the parametrium containing the uterine artery, artificially separated from the surrounding mass." He agrees that the parametrium constituting the ligamentum transversum colli is of great importance physiologically, in that they fix the cervix and form the most fixed point of the uterus. In a brief summary he states that: "the whole structure is nothing but an inseparable continuation of parametrium which surrounds and fixes the cervix." He considers the utero-sacral ligaments as small folds of peritoneum, and contain nerves, perineural connective tissue and smooth muscle; they carry the nervi erigentes, pushing their way through the mesodermal tissue from their points of emergence from the anterior sacral foramen.

b. Descriptive and topographical

The descriptive and topographical statements by Cunningham (19) of the connections of the uterus and its relations to the peritoneum laterally and posteriorly may be taken as the consensus of opinion among anatomists. The deep pouch between the uterus and vagina in front and the rectum behind is called the pouch of Douglas (excavatio recto-uterina) and its entrance is bounded on each side by a crescentic peritoneal fold, which passes from the posterior surface of the cervix uteri to the posterior wall of the pelvis, and ends near the side of the rectum. These crescentic folds are called the recto-uterine folds (plicae recto-uterinae), and each contains between its layers a considerable amount of fibrous and smooth muscular tissue. Some of these fibers, which are continuous with the uterine wall, pass backward to reach the rectum and constitute the recto-uterine muscle (ligamentum sacro-uterinum). In many cases the recto-uterine folds become continuous with one another across the middle line behind the cervix uteri, and form a transverse ridge termed the torus uterinus.

A comparison of Morris (20), Piersol (21), and Gray (22) with Cunningham (19) and others, inevitably leaves the student in doubt as to what really constitutes a sacro-uterine ligament and its relation not only to the plica recto-uterina but the musculus recto-uterinus as well.

Morris (20) calls the posterior peritoneal folds of the uterus, 'the recto-uterine ligaments,' and states that they become continuous with the peritoneal investment of the second part of the rectum, and that between their layers lie the utero-sacral ligaments, the latter being flat fibro-muscular bands, extending from the highest part of the cervix uteri, where they are more or less continuous with the uterine fibers in the recto-uterine peritoneal folds, to the sides of the sacrum



Fig. 1 Semi-diagrammatic drawing of female pelvic viscera viewed from above, uterus and adnexa being drawn forward and vessels projected against peritoneum.

opposite the lower border of the sacro-iliac articulation. They run one on each side of the rectum near the junction of the first and second parts. Their muscle fibers (the recto-uterine muscles) become continuous with those of the rectum posteriorly and more anteriorly they lie in the recto-uterine peritoneal folds, which form the lateral boundaries of the pouch of Douglas.

Piersol (21) states that between the layers of the folds (plicae-recto-uterinae) robust bundles of fibrous and smooth muscular tissue

extend from the uterus to be inserted partly into the rectum, these constituting the utero-rectal muscle, and partly into the front of the sacrum as the utero-sacral ligament.

Gray (22) says that the sacro-uterine ligaments (*plicae-recto-uterinae*) are contained in the peritoneal folds of Douglas. They pass from the second and third bones of the sacrum, downward and forward on the lateral aspects of the rectum to be attached one on each side of the uterus at the junction of the supra-vaginal cervix and the body. They contain fibrous tissue and unstriated muscle fibers. Muscular fibers from the uterine wall to the rectal wall constitute the recto-uterine muscle. This muscle is part of the sacro-uterine ligaments.

IV. EXPLANATORY REMARKS

The terms cardinal ligament, *ligamentum transversale colli uterini* and suspensory ligament are synonymous. The parametrium includes all of the fibro-elastic tissue lying lateral to the uterus; the paravaginal tissue that which lies opposite the vaginal vault; while the term paraplical will likewise signify the tissue lateral to the recto-uterine folds, or more specifically that lying lateral to the recto-uterine fossa and fibrous tunic of the rectum. It fills the subperitoneal cavity as defined by Moritz.

The sacro-uterine and recto-uterine folds are the same structurally, the only difference being the manner of termination, which makes it necessary from a physiological standpoint to speak of them separately.

The term fibro-elastic will be used instead of Cameron's (13) term perivascular tissue, because the term perivascular defines only a part of the parametrial supporting tissue, and its function is not primarily to support blood vessels as will be explained later.

V. COMPARATIVE SERIES

In animals, which habitually assume the horizontal position in locomotion, there is to be found a simpler and less condensed condition of the uterus as regards bulk than in those animals which assume the erect position. Such animals possess either two uteri or a uterus *bicornis*. The uteri are therefore comparatively less bulky and the weight is distributed along a more extensive peritoneal attachment. Such attachment usually ex-

tends as far cephalad as the caudal pole of the kidneys. At all times, except during short periods of time when other than the horizontal position is assumed, the weight of the uterus, distended bladder and rectum, cause these organs to fall ventrad in the hypogastric region of the abdominal cavity. The uterus in a state of physiological rest is relatively light and consequently there is less need of true or fibrous ligaments, such as are present in the higher Primates, where the uterus is more con-

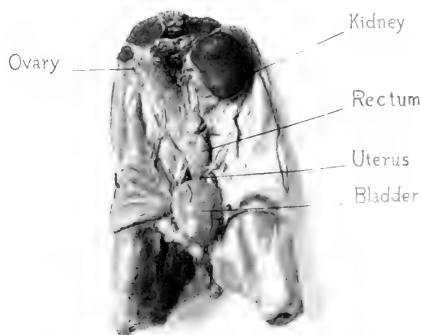


Fig. 2 Photograph showing relations of rectum, bladder and extensive peritoneal attachment of uterus in an animal which assumes the horizontal position in locomotion. Dog (*Canis familiaris*).

centrated in bulk and weight, and the erect position is assumed habitually, except during limited periods.

Therefore, in animals possessing a uterus bicornis, the peritoneal reflections are sufficient with a very meager amount of parametrial tissue to maintain the normal position of the organ. In the small series of animals examined during the preparation of this paper, one very important and significant fact was determined, and that is, that there were always bundles of smooth muscle fibers present in the peritoneal folds passing dorsad from the vagina to the rectum. Laterally, in the parametrial and paravaginal tissue, such bundles were practically

absent and the fibro-elastic bundles were reduced to a minimum. It is therefore logical from an evolutionary standpoint to conclude that the presence of the recto-uterine muscle in these folds appears at an early date in the vertebrate series, and that they possess a phylogenetic significance.

1. GUINEA PIG

In the guinea pig, two distinct peritoneal folds pass dorsally from the sides of the vagina to the sides of the rectum. They

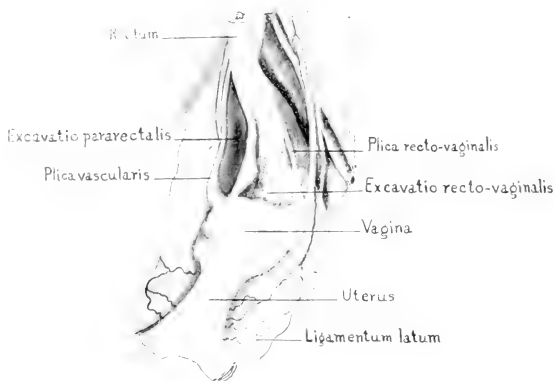


Fig. 3 Drawing showing rectum, vagina, uterus, recto-vaginal pouch and recto-vaginal folds in guinea pig (*Cavia cabayia*).

are therefore recto-vaginal folds, and must be termed the lateral folds to distinguish them from the median recto-vaginal reflection of peritoneum at the bottom of the recto-vaginal fossa. Lateral to the rectum and recto-vaginal fold is the pararectal fossa, which is in turn bounded laterally by a peritoneal fold conveying utero-vaginal blood vessels. The lateral recto-vaginal folds and broad ligaments of the uterus are extremely thin and more or less transparent to translucent. By means of serial microscopical sections bundles of smooth muscle fibers

have been traced from the uterus toward the rectum, in the recto-vaginal folds. These delicate muscular fasciculi are continuous with the noticeable longitudinal fibers on the dorso-lateral facies of the uterus. It is important to note that the stratum fibrosum of the peritoneum shows marked thickening. The few muscular fasciculi that do reach the sides of the rectum may be traced along its lateral wall to be inserted into its fibrous sheath, or continuing dorsally, become lost in the mesorectum. Many of the fasciculi diminish in size as they pass through the folds, their fibers apparently becoming inserted into the peritoneum, but their actual termination has not been observed. Between the two peritoneal layers of the recto-vaginal folds there is a small amount of extremely fine areolar tissue with a few fibro-elastic filaments. In the parametrial and paravaginal tissue there is a very meager amount of fibro-elastic elements. The cephalic extremity of the vagina is freely movable and lies between the bases of the broad ligaments. The recto-vaginal folds varied quite a good deal in size and symmetry in the different guinea pigs examined.

2. BELGIAN HARE

Distinct recto-vaginal folds are present. These and the broad ligaments are thin and more or less transparent, containing a varying amount of adipose tissue. Visible fibers are very sparse in the parametrial and paravaginal tissue. The free margins of the recto-vaginal folds are slightly thickened and opaque, as in the guinea pig. This opacity is due to temporary aggregation of the muscular fasciculi. The stratum fibrosum of the peritoneum constituting the folds, shows distinct thickening and at its deep surface passes insensibly into the extremely delicate areolar tissue between the layers, where also a few fibro-elastic bundles are found. In the parametrial and paravaginal tissue, there is a varying paucity of fibro-elastic bundles, but no fibers that could be taken to constitute potential ligaments, or noticeable aggregations of fibers to constitute true ligaments are present (fig. 5). The fasciculi of the recto-uterine muscle vary

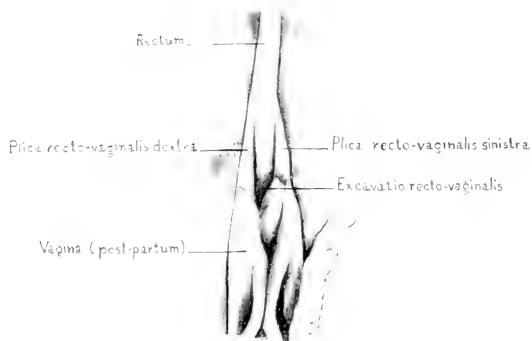


Fig. 4 Drawing showing rectum, vagina (post-partum) and recto-vaginal folds in Belgian hare (*Lepus europeaus*).

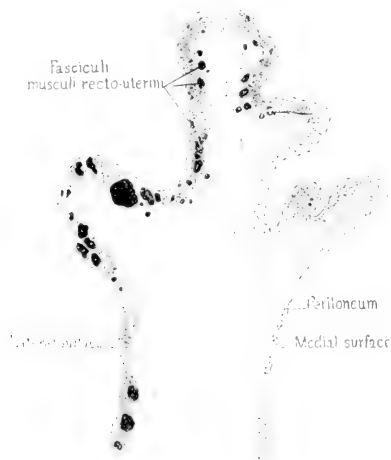


Fig. 5 Transverse section of a lateral recto-vaginal fold at middle third, showing distribution of fasciculi of recto-uterine muscle. Belgian hare. $\times 62$. Reduced to one-half.

greatly in size and are distributed chiefly in the apical and lateral peritoneal layers of each fold. Many of the fasciculi appear to be almost submesothelial, others which are in the minority appear to be on the inner border-line of the stratum fibrosum, the majority however are in the more central parts of that layer. The muscular fasciculi are abundant in the vaginal extremity of the recto-vaginal fold, but become greatly diminished or absent in the rectal extremity of the same. A large percentage of the fasciculi therefore terminate in the fold probably by insertion into the more superficial part of the stratum fibrosum. Their actual termination has not been observed.

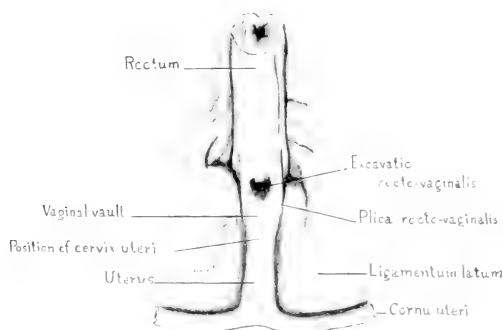


Fig. 6 Rectum, vagina and uterus of cat (*Felis domestica*), showing recto-vaginal folds.

3. CAT

The recto-vaginal folds are thicker and relatively smaller than in the hare, and pass more noticeably upon the ventral surface of the rectum; they are transparent as are also the broad ligaments. The recto-uterine muscles form distinct bands and attain the sides of the rectum; they are fusiform in transverse section and each carries its own blood-vessels. The stratum fibrosum of the peritoneum is distinctly thickened and contains the muscular fasciculi. The fasciculi are most abundant and

largest in the vaginal extremity of each fold, but many reach the side of the rectum and continue upon the same for a varying distance. They are not all in the prominent part of the fold on the rectum, for as the peritoneum spreads out the fasciculi necessarily go with it. The recto-vaginal folds vary in prominence and the distance to which they extend cephalad on the rectum depends on the degree of distention of the latter. It has

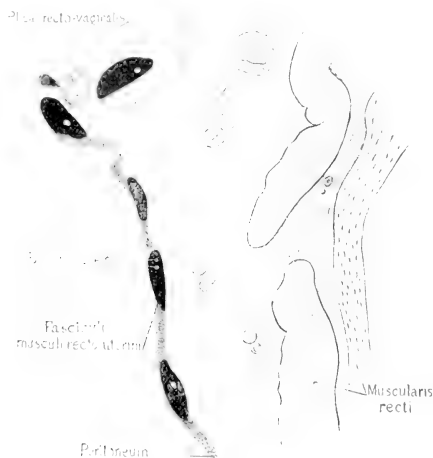


Fig. 7. Semi-diagrammatic drawing of lateral wall of rectum of cat, showing peritoneum and fasciculi of recto-uterine muscle, recto-vaginal folds and muscularis recti (Transverse section). $\times 175$. Reduced to one-third.

been observed that the peritoneum is anchored to the rectum by fibrous trabeculae which arise in the intermuscular fibrous tissue of the muscularis, which by passing outward, through intervals in the longitudinal layer become inserted into the stratum fibrosum.

Figure 8 shows the details of a muscular fasciculus, and its more or less centrally located blood vessel. In the majority of cases the vessels are intra-fascicular, but they vary in position to the periphery and may be supra-fascicular.

4. DOG

In the dog, the anatomical relations between the pelvic viscera differ considerably from those in the series described above. The vagina during the inter-estral period is rather firmly fixed at its cephalic extremity. The bladder has a short but distinct neck, the peritoneum being reflected from the cervix vesicae upon the anterior vaginal wall before attaining the uterus. In the individuals examined small single or double recto-vaginal folds passed from the utero-vaginal junction to the rectum. The

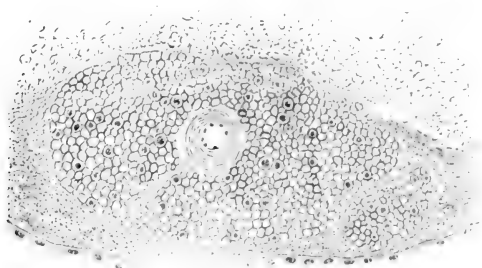


Fig. 8 Detailed camera lucida drawing of a fasciculus of recto-uterine muscle with its blood vessel, showing relation to peritoneum (Transverse section). Cat. $\times 235$. Reduced one-half.

ligaments of the bladder and uterus are thin, transparent and contain a varying amount of adipose tissue. The bladder possesses well developed dorso-lateral ligaments which suspend it from the dorsal pelvic wall. The uterus is held in close relation to the ventral surface of the rectum, by its relatively large broad ligaments which pass from the sides of the cervix and cornua, each crossing the dorso-lateral ligament of the bladder of its own side. The recto-uterine muscles pass nearly directly between the two organs, and in the small single or bilateral recto-vaginal folds; or when one or both are absent by way of the peritoneum at the bottom of the recto-vaginal fossa. There is a broad recto-uterine space. Between the folds of the broad liga-

ments and in the parametrium there is relatively a little more fibro-elastic tissue, but no aggregations which might be construed as constituting true or potential ligaments.

5. GENERAL CONSIDERATIONS

From the study of the above comparative series the following facts are to be noted. Distinct recto-vaginal folds pass from the dorsal wall and cephalic extremity of the vagina to the rectum, the former being more or less free and movable; that these

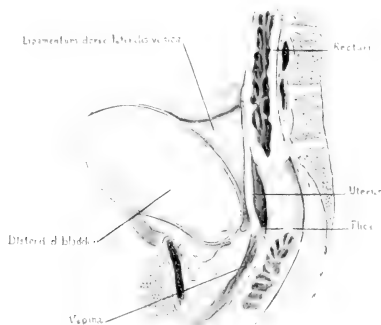


Fig. 9 Drawing of sagittal section of pelvis of a bitch showing relations of viscera and peritoneal folds.

recto-vaginal folds vary in size, and that they can be increased by traction on the uterus or more so by traction on both uterus and rectum. The stratum fibrosum of the peritoneum has in all cases undergone thickening or hypertrophy, and the fasciculi of the recto-uterine muscle are contained in it. The fasciculi of the latter are most abundant at the vaginal extremity of the fold and diminish in size and number as the rectum is approached, in some cases not reaching it. Some of the fibers of the muscular fasciculi are apparently inserted into the stratum fibrosum of the peritoneum, fewer of the bundles possibly being inserted into the fibrous tunic of the rectum or lost in the mesorectum.

The fibro-elastic tissue constituting the parametrial, paravaginal and paraplical tissue is very scanty in amount and does not form aggregations to constitute so-called true ligaments, nor in sufficient quantity to form potential ligaments. The body of the vagina is more firmly attached than the cephalic extremity.

VI. PRIMATE SERIES

1. MONKEY (MACACUS)

In the monkey, the uterus is undivided and the anatomical conditions are similar to those in the human female. The weight of the uterus is localized and therefore calls for stronger mechanical support. There is marked increase in the amount of the parametrial, paravaginal and paraplical fibro-elastic tissue, which can be recognized as constituting potential ligamentous aggregations. The specimens examined had unfortunately been embalmed in the usual way, but nevertheless the material demonstrated that the structure and relations were similar to those in the human female. Recto-uterine or sacro-uterine folds were absent, although potentially present. A short plica was present on each side at the utero-vaginal junction. These folds are not always present in woman, but can always be demonstrated as being potentially present, for traction forward on the uterus brings them into prominence. The thickened peritoneum with the recto-uterine muscles are always present.

The histological examination of the material from the monkey was not satisfactory as to details, on account of the amount of cytolysis and softening of the tissues generally, but everything indicated similar structural conditions as in woman.

2. HUMAN

For the purpose of establishing a point of departure, the pelvic structures in a state of full maturity or development in woman, will first be taken up, followed by those before maturity and, lastly, those in the stage of senility.

a. Examination of the structures in a recent post mortem state

The careful dissection of the pelvic viscera of a woman 35 years old gave the following results: The recto-uterine folds were moderate in prominence and diminished gradually, to become lost in the general plane of the peritoneum without attaining the posterior pelvic wall. The rectum was median in position and without a mesorectum opposite the third sacral vertebra.

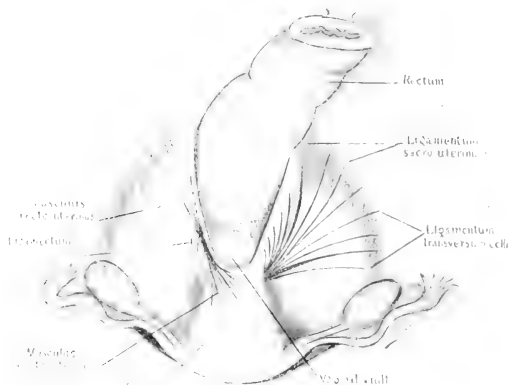


Fig. 10. Semi-diagrammatic drawing of female pelvic viscera viewed from above, uterus and adnexa having been drawn strongly forward. Position of potential ligaments are shown in black lines.

Traction forward on each sacro-uterine fold separately, tightened the peritoneum and raised its surface into a series of small ridges, that radiated toward the sacro-iliac junction and lateral border of the promontory, from the point where the fold disappeared, in a direction downward and inward to the middle of the second sacral vertebra at the edge of the rectum and reflection of the peritoneum upon it. The area raised on the right was triangular but did not extend so far laterally as given in figure 10; on the left side the raised area was narrower and less triangular than on the right. Traction backward on each fold

tightened the peritoneum about the uterine cervix and vaginal vault, raised the cervix upward and backward, forced the fundus against the bladder and raised the vaginal vault.

Traction on the uterus to the right or left simply tightened the broad ligaments, peritoneum, and subperitoneal fibro-elastic tissue without any special result.

Bodies embalmed in the usual way for dissection, or with a 10 per cent to a 40 per cent formaldehyde solution, will usually show the same result of traction on the sacro-uterine or recto-uterine folds, and parametrial fibro-elastic tissue as above stated. The embalming fluids used, especially the formaldehyde solution, produced marked contraction of fibro-elastic tissue and such contraction produces bands which are rendered slightly prominent as peritoneal ridges, more or less above the general surface contour of the peritoneum, the results varying in different cadavers. Two female cadavers in the anatomical collection of the university show these facts beyond doubt.

Traction per vaginum downward and forward on the uterus depresses the sacro-uterine or recto-uterine folds and draws on the sacral attachments of the peritoneum, in the line of the folds. At the same time there is a coincident appearance of a prominent ridge in each dorso-lateral wall of the vaginal vault, corresponding to the position of the sacro-uterine and plico-vaginal ligaments. There is a tightening of the subperitoneal tissue laterally as well as dorso-laterally to the uterus.

Reflection of the peritoneum lateral to the uterus and recto-uterine fold, uncovers a mass of fibro-elastic tissue, through which run the branches of the hypogastric vessels, lymphatics, and nerves on their way to the uterus, vagina and bladder. Traction in different directions on this mass demonstrated its great elasticity as well as the intrinsic movements of the fasciculi and lamellae over each other. Careful dissection and examination with a moderately strong hand lens clearly defined a mass of interlacing fibers and fasciculi ensheathing the vessels and nerves and having an attachment to the fascia covering the levator ani, coccygeus and obturator muscles, as well as the pre-sacral fasciae and peritoneum. Pulling on the fibro-elastic

tissue from different points laterally and at the periphery, drew the uterus in the same direction; drawing the uterus toward the opposite side of the pelvis tightened the fibro-elastic mass which appears to radiate chiefly from the sides of the uterine cervix, while traction toward the middle of the inguinal ligament tightened the fibro-elastic mass laterally and posteriorly on the opposite side.

The examination of several autopsy specimens not only verified the above facts but revealed others (fig. 10). By carefully cutting through the peritoneum in the anterior part of the lateral wall of the recto-vaginal fossa, dissecting out the areolar tissue filling in the meshes of the fibro-elastic net-work and isolating a number of fibers, a dimpling of the peritoneal surface along the sacro-uterine fold could be produced by pulling downward on the fibers. Other fibers appeared to be attached further posteriorly in the vicinity of the sacrum. Pulling upward on the same fibers raised the vaginal vault or the cervix uteri. These experiments determined that there were two sets of fibers which entered or passed beneath the fold. One set passing from the sides of the uterine cervix backward, giving off fibers which were inserted into the stratum fibrosum of the peritoneum along the line of the fold, and others reaching the presacral fascia. The second set passed from the sides of the vaginal vault below the above and inserted into the stratum fibrosum along the anterior two-thirds of the fold. The study also determined that the meshes of the fibro-elastic network varied in size and irregularity. At times there was observed an apparent increase in the density of the fibro-elastic mass opposite to the cervix uteri and vaginal vault. At the periphery of the pelvic cavity just in front of the sacro-iliac articulation, the network appeared less dense. This condition was not always evident.

In cadavers that have been embalmed in the usual way and dissected, there will be found fasciculi passing laterally from the side of the uterine cervix, and forming a more or less distinct band which corresponds in position to the cardinal ligament or ligamentum transversale colli. Other fasciculi which pass

backward beneath the sacro-uterine fold to reach the presacral fascia, correspond to the course of the so-called sacro-uterine ligament. The fibers sweeping downward from within the anterior two-thirds of the sacro-uterine fold to be inserted into the sides of the vaginal vault, are termed the 'plico-vaginal ligament.'

b. Histological observations. Fixed material

1. *Adult 35 years old.* Figure 11 is a photomicrograph of a portion of a transverse section of the sacro-uterine fold of a

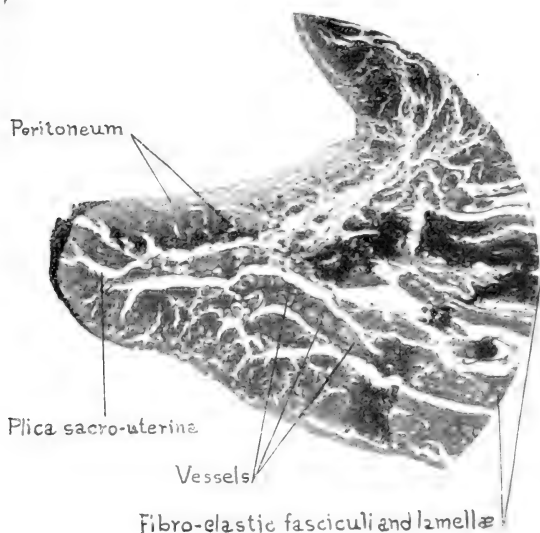


Fig. 11 Photomicrograph of a transverse section of recto-uterine fold at middle of anterior third, of a woman 35 years old. $\times 50$, oc. 2; obj. A.

woman 35 years old. The section is through the anterior third of the fold and includes the vaginal vault. The stratum fibrosum of the peritoneum is thickened and dense, the muscular fasciculi are abundant within it.

The inner face of the dense fibrous layer shows a distinct tendency to break up into segments or divisions and most of them appear to be directly continuous with the fibro-elastic fasciculi or lamellae which sweep downward out of the fold to become part and parcel with the general fibro-elastic and perivascular network. Beneath the peritoneum therefore, the fibro-elastic

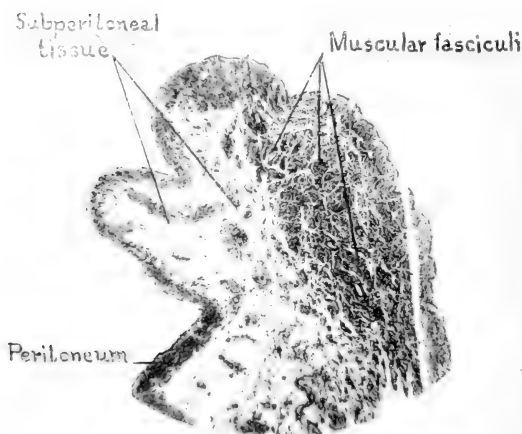


Fig. 12 Photomicrograph, transverse section of right recto-uterine fold in anterior part of middle third, of a child 10 years old. $\times 30$, oc. 4; obj. A² (Zeiss).

tissue is dense and rich in blood vessels, lymph vessels and nerves. These fibro-elastic fasciculi and lamellae are parts of the plico-vaginal and sacro-uterine ligaments. The plico-vaginal constituents end in the inner surface of the thickened peritoneum, and are the fibers which caused the dimpling in of the peritoneal surface of the fold in the experiments reported above.

2. *Child 10 years old.* In sections (fig. 12) made at the middle of the sacro-uterine folds the peritoneum is thickened as usual and small fasciculi of muscle fibers are scattered sparsely through the slightly dense stratum fibrosum. On the border line between the latter and the subperitoneal tissue there is an aggregation of numerous large muscular fasciculi, small vessels and nerves. These fasciculi are surrounded by rather loose fibro-elastic tissue, but the general character of it is such that it can be associated with the peritoneum, rather than with the subperitoneal tissue. In all probability, as growth continued, a

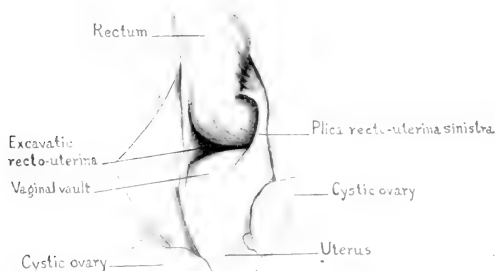


Fig. 13 Drawing showing the female pelvic viscera in an infant 40 days old. The uterus has been drawn forward. Made from the specimen studied.

greater condensation would take place and these elements would become more closely bound to the peritoneum. The sections are from behind the plico-vaginal ligament, and there are no fibers sweeping downward which could be taken for it. It is possible that a part of the above mentioned muscular fasciculi are those that accompany the sacro-uterine ligament.

3. *Infant 40 days old.* Figure 13 is a drawing of the pelvic viscera of an infant 40 days old. The specimen was cut in serial transverse sections. The left sacro-uterine fold was very prominent and terminated lateral and dorsal to the rectum. The right fold was much less prominent and terminated upon the lateral

wall of the rectum. The structure of the folds as regards density of the tissues, was similar to those of the adult 35 years old.

Figure 14 is a photomicrograph of a section through the middle of the right fold, the peritoneum is thickened, and in the flattened apex of the fold the intraplical fasciculi are very intimately connected to the deep surface of the stratum fibrosum;

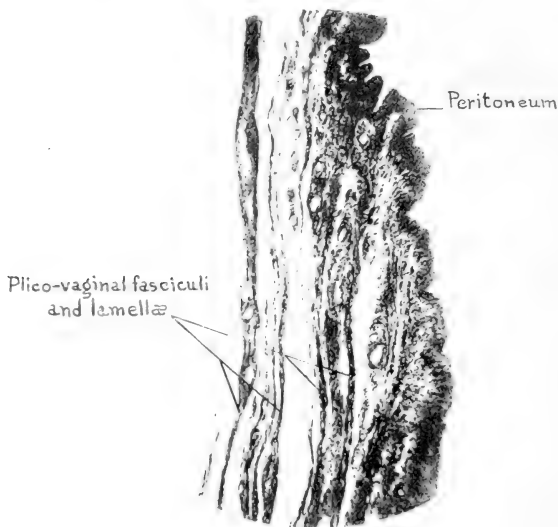


Fig. 14 Photomicrograph of a transverse section through the middle of the right recto-uterine fold, of an infant 40 days old. Note the dimpling of the peritoneal surface. $\times 50$, oc. 4; obj. A.

in the latter there are small muscular fasciculi. From their points of insertion into the peritoneum, the fibro-elastic fasciculi stream downward lateral to the recto-uterine fossa and become continuous with the general perivascular tissue.

The photomicrograph shows the wrinkling of the peritoneal surface and the fibro-elastic fasciculi can be definitely traced downward from their attachment to the stratum fibrosum. In

both folds, these fasciculi are plico-vaginal and sacro-uterine, the former being the most evident.

The intraplical muscular fasciculi are those which in part probably accompany the sacro-uterine fibro-elastic fasciculi. To what extent these muscle bundles become associated with the peritoneum to constitute recto-uterine muscle bundles is not known.

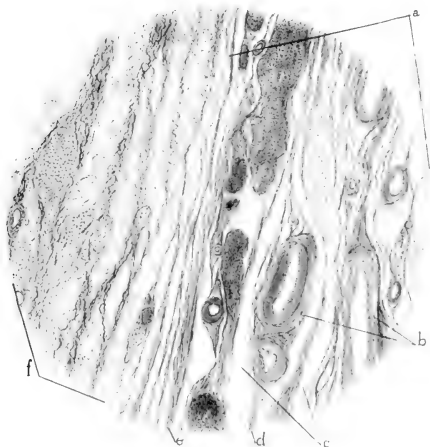


Fig. 15 Photomicrograph of a transverse section of a right plica recto-uterina at anterior third, through plico-vaginal ligament (*a*), showing perivascular (*b*) character of fibrous tissue, meshes (*c*) between the lamellae (*d*) and fasciculi (*e*) filled with delicate areolar or adipose tissue (the former has not been filled in); lateral to ligament the tissue is distinctly fibro-elastic and areolar (*f*). Infant 40 days old. $\times 88$ diam., oc. 2; obj. A. Reduced to one-third.

Figure 15 is a drawing of the paraplical tissue below the point shown in figure 14. It shows the perivascular character of the fibro-elastic fasciculi and lamellae. The tissue shown here is a part of the plico-vaginal ligament. Particular attention should be given to the manner in which the mesh-work is formed—by division and union of fasciculi and lamellae and

how they enclose the vessels. The whole is representative of the arrangement throughout the parametrial, paravaginal and paraplical network. It has been observed that the lymphoglandulae are not as a rule enveloped by the fibro-elastic lamellae, but project into a mesh, being only attached at the hilus to a lamella, which immediately envelops the vessels entering or leaving it. At least this has been the case in a number of instances. The capsule of the gland is continuous with the lamella at the hilus.

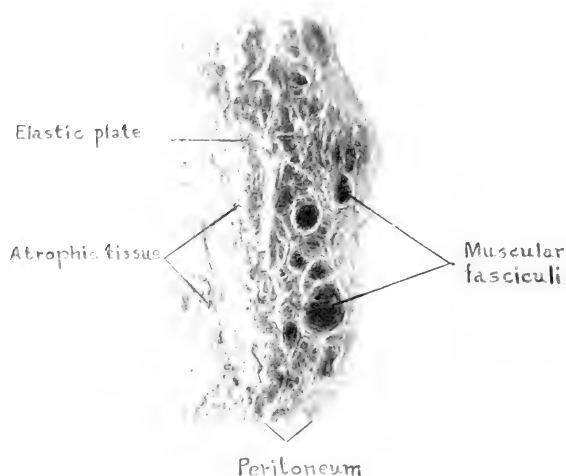


Fig. 16. Photomicrograph of a transverse section of a sacro-uterine fold at the posterior third, of a woman 65 years old. $\times 50$, oc. 2; obj. A.

The meshes of the network are filled with delicate areolar or adipose tissue, as shown in figure 15, where a part of the meshes have not been filled in, to better demonstrate the fibro-elastic network. Lateral to the plico-vaginal fasciculi, the tissue is distinctly elastic and areolar.

4. *Adult 65 years old.* Figure 16 is a photomicrograph of a portion of a transverse section of a plica sacro-uterina of a woman

past the menopause. The peritoneum still remains much thickened, the bundles of the recto-uterine muscle are very distinct, and the subperitoneal fibro-elastic tissue shows distinct senile atrophy. The general looseness of the tissue is noticeable and adipose tissue is more abundant than at the other ages considered above.

Figure 17 is a drawing of a portion of the peritoneum in the section from which figure 16 was made, but more highly magnified. The muscle bundles have been drawn in heavy black to

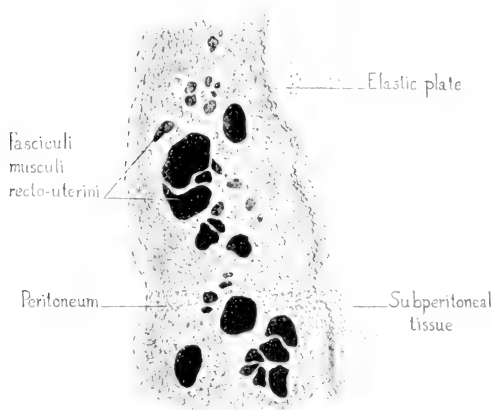


Fig. 17 Photomicrograph of part of a transverse section of plica sacro-uterina at posterior third, showing distribution of fasciculi of recto-uterine muscle, elastic plate and subperitoneal connective tissue in a woman 65 years old. $\times 88$. Reduced to one-third.

emphasize their abundance. At the inner limit of the dense stratum fibrosum there is a distinct layer or plate of elastic tissue. Figure 18A shows this layer as brought out by Weigert's elastic tissue stain. This elastic plate lies to the inner side of the thickened fibrous stratum of the peritoneum, and becomes much thinner at the periphery of the plical area where it approaches quite close to the mesothelium; it appears to limit internally the stratum fibrosum. Whether or not this layer will be of value in

determining the relation of the recto-uterine muscle to the peritoneum remains to be seen. Figure 18B illustrates the abundance of elastic fibers in a fasciculus of the recto-uterine muscle of the same section.

5. *Adult 83 years old.* Figure 19 shows one of a transverse series of sections through a sacro-uterine fold of a woman 83 years old. The section has been taken from the fold at the middle of the middle third. The fibrous stratum of the peritoneum is thickened as usual, and immediately beneath the mesothelium there is a distinct layer of undulating fibers (A), that separates the stratum containing the muscular fasciculi from the mesothelium. The fibers of the deeper layer (B) are more irregular and broken, receiving the insertion of the



Fig. 18. A. Section of peritoneum from sacro-uterine fold of a woman 65 years old. B. Transverse section of a recto-uterine muscle, bundle, showing abundance of elastic tissue. Weigert's elastic tissue stain. $\times 90$ diam. Camera lucida drawing. Reduced one-half.

plico-vaginal fibers which are well shown in the sections. The fibro-elastic tissue is less compact than in the infant 40 days or the woman 35 years old on account of the senile atrophy present. Weigert's elastic tissue stain also brought out a greater irregularity in the distribution of the elastic fibers. The elastic plate so well defined in figure 17 is missing here, although a very irregular line of elastic fibers is present. The stain has also brought out a greater amount of elastic tissue in the submesothelial fibrous layer that was not noticeable in the sections from which figure 16 was made. In figure 18B there is shown an abundance of elastic fibers in relation with the muscular fasciculi and it is to be noted that they are between the elastic plate

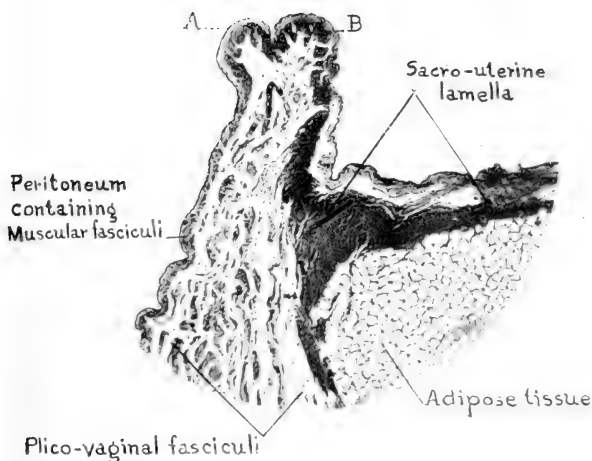


Fig. 19 Photomicrograph of a transverse section of sacro-uterine fold at middle of middle third, in a woman 83 years old. $\times 30$, oc. 2; obj. a^2 (Zeiss).

and the mesothelium. Beyond the area of distribution of the fasciculi of the plico-vaginal and sacro-uterine ligaments, adipose tissue is very abundant, scattered through which is a varying but meager number of fibro-elastic lamellae that have been cut across.

VII. VARIATIONS

It is well known from observations made in the dissecting room and at autopsies, that the peritoneal surface contour of the pelvic cavity and relative size of the pelvic viscera are subject to considerable variation; at times to marked asymmetry. The bones forming the pelvic wall may vary so as to render the cavity narrow and relatively deep, or broad and relatively

shallow. The muscles may be much better developed in some individuals than in others, and the amount of fibro-elastic tissue may likewise vary.

Every gynecologist knows that the perineal body may be large and strongly constituted in some women, or feeble and scarcely recognizable. The muscles guarding the pelvic outlet are very strong and capable of affording ample support to the pelvic organs in some individuals, in others they are poorly developed and incapable of withstanding continued strain. These variations bespeak for a general habitus which may indicate a predisposition to the persistent maintenance of the norm, or an easy deviation from the same. The plicae sacro-uterinae are no exception to the rule. In certain cases they are strongly developed and very prominent and symmetrical or asymmetrical; at other times they apparently are entirely absent, or a short plica may be present on each side near the uterus. But even when visibly absent, they are potentially present, for forward traction on the uterus produces them. Or a distended rectum, by carrying the peritoneum away from the pelvic wall, will produce them or convert sacro-uterine into recto-uterine folds. The position of the rectum also influences the character of the folds. If the rectum is median in position, both folds are usually sacro-uterine; if that organ is sinistral, the corresponding fold will be recto-uterine and the opposite will be sacro-uterine and vice versa when the rectum is dextral in position. Histological examination reveals similar variations, both as regards quantity and symmetry, in the uterine supports.

VIII. EXPERIMENTS

In an anesthetized cat, with the abdomen opened and the pelvic organs exposed to view, the uterus and bladder have been observed to contract more or less intermittently as peristaltic waves passed down the rectum. Though slight, these contractions were unmistakable.

In the animal studied, the rectum happened to be strongly distended with fecal matter opposite the recto-vaginal fossa and the point of attachment of the recto-vaginal folds. The

viscus was stretched in the directions of both its primary and secondary axes. The recto-vaginal folds were large and exhibited occasional contractions, which feebly raised the vaginal vault. After the sigmoid colon had been severed and the fecal contents expelled, it was observed that the intestine slowly contracted and sank lower in the pelvis. During this descent the recto-uterine folds diminished in size and the vaginal vault sank carrying the uterus with it. The rectum was then injected to reproduce the effect on the vaginal vault through traction on the recto-vaginal folds. The rectum was clamped behind the free extremity of the vagina, and the rectum slowly distended with water. As a consequence, the rectum pulled cephalad on the folds raising the vaginal vault and carrying the uterus toward the abdominal cavity. These observations in the cat suggested, first, that the distended rectum mechanically raises the vaginal vault when its fecal contents are passing caudalward and thus prevents compression of the organs of reproduction; second, the act in all probability excites a reflex contraction in the uterus which participates in the act through the recto-uterine muscles; third, that the uterus can automatically raise itself through contraction of the recto-uterine muscles in the recto-vaginal folds.

These experiments were repeated on a cadaver of an infant 40 days old. The uterus was raised as before by traction on the right recto-uterine fold by distention of the rectum, and at the same time the partial conversion of the left sacro-uterine fold into a recto-uterine fold was accomplished. Hence the uterus can automatically raise and tilt itself ventrad in primates, or be raised mechanically by the recto-uterine folds when the rectum is distended. Or, distention of the rectum may cause reflex contractions of the uterus. When the sacro-uterine folds are present and the rectum is median in position, the uterus must act alone through a reflex. If the rectum is sufficiently distended, it can convert sacro-uterine folds into recto-uterine folds. When the uterus is raised through traction or contraction of the recto-uterine muscle, the sacro-uterine fold is tightened, the plico-vaginal ligament is pulled upward carrying the vaginal vault with it.

IX. THE MUSCULI LEVATORES UTERI

No reference has been made to the muscular tissue that passes off laterally from the uterus to be dispersed through the fibro-elastic tissue filling the subperitoneal space lateral to the uterus. Microscopical examination of the parametrial tissue shows that smooth muscle is abundant about the point of insertion of the fibro-elastic tissue into the sides of the uterine cervix, and that it diminishes in abundance as it is traced laterally and posteriorly. The fibers appear to be inserted into the perivascular tissue, the fibrous fasciculi and fasciae of the nearby muscles, but their actual termination has not been determined. Direct continuity of the recto-uterine muscles with the muscularis uteri is established. This muscular tissue extends from the uterus into the adjacent tissue at an early embryonic period. These muscles, one on each side of the uterus, enable that organ to automatically raise itself on the fibroelastic suspensorium. By their more or less intermittent contractions, they probably become one of the chief agents in aiding the venous circulation in the peri-uterine venous plexuses; the pelvic diaphragm being accessory to the act.

X. DISCUSSION

In taking up the discussion of the uterine ligaments, it is necessary to consider briefly the mechanical supports of the uterus in order to fully appreciate the part which the fibro-elastic or so-called perivascular tissue plays in the process.

The mechanical supports of the uterus will be considered in the following order:

1. The levator ani and its superior fascia.
2. The peritoneum.
3. The fibro-elastic tissue filling in the interval between the two first mentioned.

Gynecological text-books usually speak of the pelvic floor as a support of the uterus. Fothergill (15) and Cameron (13), the former preceding the latter, have described the levatores ani as forming 'a tunnel' on each side of the vagina and being attached

to it at its lower part. Cameron (13) is quite right when he states that the uterus which is above the vagina—the latter not receiving any direct support in its upper part from the muscular diaphragm—has no support from these muscles. The superior fascia of the levatores ani does not prevent the descent or ascent of the uterus. He asks why it is that the superincumbent intestines do not crowd the uterus and the vagina downward concertina-like? Cameron (13) rightly concludes that some other structures prevent this, which according to him are the 'perivascular fascia' and the blood vessels.

It is certain that the pelvic diaphragm plays a large part in supporting the pelvic viscera, and indirectly the descent of the superincumbent intestines, which when normally suspended by their mesenteries exert very moderate pressure. The normal supporting function of the pelvic diaphragm may be likened to a foundation upon which a superstructure rests, and which when it ascends or descends, carries the superstructure with it. By virtue of its contractile power and up and down movement, it also aids in preventing venous stasis within the pelvic plexuses, besides fulfilling other functions.

The superior fascia of the levatores ani steadies and aids in maintaining the cephalic extremity of the vagina and uterus in their median position, and also gives support and attachment to the parametrial fibro-elastic mesh-work. The peritoneum may be regarded as a support in the sense that it envelopes the pelvic organs as a sheet, forming lateral folds for the support of vessels and the adnexa uteri, maintaining the uterus in its normal anatomical position, and preserving this relation in its physiological excursions. Besides, as Cameron (13) states, it furnishes the superior attachment of the 'perivascular tissue.' The sacro-uterine or recto-uterine fold of the peritoneum has a varying degree of usefulness, which has already been partially stated and will be referred to again. Cameron (13) denies that these folds exert any supporting influence on the uterus, but laid great stress on the 'perivascular tissue,' which is weakest anteriorly lateral to the bladder, and between the folds of the broad ligaments; but increases in thickness as it is traced back-

ward, and is greatest in amount opposite the broad ligaments and the sacro-uterine folds. Its attachments, to quote Cameron (13) again, are as follows: "Above to the peritoneum, below to the pelvic sheaths of the levator ani and coccygeus, externally to the obturator fascia, and higher up to the periosteum of the innominate bone, while internally it blends with the connective tissues of the viscera. Its attachment to the lateral pelvic wall is rendered further secure by the fact that the parietal branches of the hypogastric vessels pierce the pelvic parietes in order to reach their respective destinations, and in doing so their sheaths blend with the surrounding bony and muscular structures." It is admitted that the "main trunks of the hypogastric vessels, in their descent on the lateral wall of the pelvis, are bound down to the latter by dense areolar tissue." Cameron (13) has somewhat over-estimated the relative size of the vessels passing to the bladder, uterus and vagina. The main vessels are large and well anchored to the lateral wall of the pelvis, and their visceral branches are supported and surrounded by the fibro-elastic tissue in part. These vessels are not a factor in the support of the uterus, and the ovarian vessels enter too high to be a support; and besides, it is to be expected that vessels which are to be put to greater or less stretching, will be more or less tortuous in their course.

The parametrial tissue contains the peri-uterine venous plexuses, which must not be kept on a strain or under continual compression. It is necessary to again call attention to the abundance of the lymphatic vascular net-work, and the numerous nerves that permeate this region. The round ligaments are of doubtful importance as uterine supports, and Fothergill (15) has pointed out that they are essentially embryonic in function. Cameron (13) has given importance to the obliterated hypogastric arteries as a pelvic support. They may act feebly. They are essentially embryonic in function. Cameron (13), after his discussion of the importance of the different pelvic supports, sums up by stating that "the perivascular fascia, plus the pelvic sheaths of the levator ani and coccygeus muscles, are the most important."

Montgomery (8) considers that the supports of the uterus are not ligaments in the ordinary sense, but consist of connective tissue, into and through which run prolongations from the uterine muscular structure, so that the organ is virtually sustained by muscular action. But he confounds the movements of the pelvic diaphragm with true intrinsic uterine activity, such as it is capable of exerting through the musculi levatores uteri. Its excursions upward and downward with 'every respiratory' movement, depends upon the action of the respiratory mechanism.

Mackenrodt (3) lays great stress on a band of connective tissue, the *ligamentum transversale colli*, as of great physiological importance in maintaining the normal position of the uterus. He recognizes that the lower opening of the pelvis is closed by pelvic fascia, which sends firm bands to the cervix and vagina; that the cervix is held fast in its embryological position by ligaments, while the uterine body is kept in position by its own weight and intra-abdominal pressure—not by ligaments. Evidently this knowledge of the 'firm bands' was acquired through the dissection of embalmed material. He says that fibers coming from the pelvic fascia to the side of the cervix are to be sharply defined from the sparse connective tissue between the folds of the broad ligament. These, he states, form a band that is the chief means of holding the uterus in position. Another statement is that this band or *ligementum transversale colli* carries the *arteria uterina* in its upper part (vide fig. 20).

Ovenden (12), in her dissections, found very "little connective tissue between the layers of the broad ligament, but at the level of the cervix, however, a thick band can be felt between the two layers of the peritoneum. It is wedge-shaped in section; the apex of the wedge is directed upward and is just above the level of the point of entrance of the uterine artery. Traced to its distal attachments, this band is found to be formed from strong fibrous connective tissue, continuous with that which surrounds the pelvic blood vessels, and also that which comes through the sacro-sciatic notch. Some of the fibers appear

also to be attached to the sides of the third and fourth pieces of the sacrum."

Mackenrodt (3) considered that the ligamentum transversale colli has its central attachment at the supra-vaginal portion of the cervix. Ovenden (12) considers it inserted partly into the vaginal vault and lateral fornix, besides into the sides of the uterus for a short distance below the point of entrance of the uterine artery (vide figure 20).

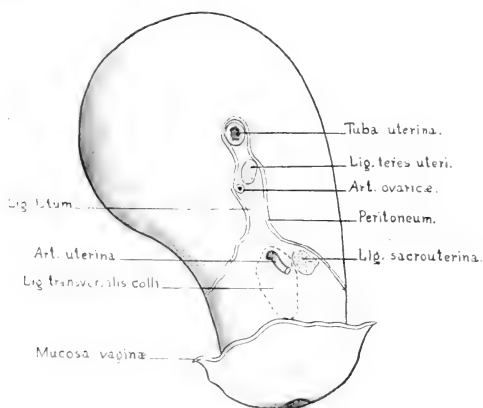


Fig. 20 Side view of uterus (as removed by vaginal hysterectomy) showing insertion of ligamentum transversale colli of Mackenrodt (after Ovenden).

These facts agree with the observations reported in the earlier part of this paper. Ovenden (12) is correct in considering that the whole mass is not inserted into the uterus, as Mackenrodt (3) asserts; and also that the sacro-uterine ligament blends with the ligamentum transversale colli near its insertion into the uterus. The plico-vaginal ligament attached to the vaginal vault becomes continuous with the sacro-uterine ligament. It is to be noted that these observations make these three ligaments continuous at their insertion into the sides of the cervix and vaginal vault.

It must be mentioned that Emmet (23) and Schauta (24) have laid emphasis on the importance of the part played by this pelvic connective tissue in maintaining the normal position of the uterus, although they did not ascribe this function to a particular band.

Specimens from the cut ends of the so-called sacro-uterine ligaments, as severed from their attachment into the sides of the uterine cervix at the time of operation for utero-vaginal prolapse in elderly women, were submitted to the writer by Dr. George B. Somers (25) for microscopical study. One ligament, evidently cut closely to the uterus, showed a great preponderance of smooth muscular tissue over the fibro-elastic; the other, much less muscular and chiefly fibro-elastic tissue. Ovenden (12) states that in microscopical sections the ligament consists largely of fibrous tissue, through which are scattered a good many bundles of smooth muscle fibers.

None of the writers have attempted to explain the marked elasticity that is inherent in the pelvic structures, not how or why the uterus can be pulled down to the introitus vulvae or to the exterior, and when released will slowly and completely return to its normal position in the pelvic cavity without further manipulation. From the experiments, dissections and observations reported in this paper, it now becomes necessary to describe the fibro-elastic suspensorium uteri, which accounts for all of the phenomena that have thus far been observed and reported.

If a square piece of thin paper be taken and folded over two or three times, cut one-half across first on one side and then on the other, when opened up it will appear as in figure 21A. Traction applied in direction of the arrows, or diagonally at the corners, will open up a mesh-work as in figure 21B. Release the paper and it will instantly return to a state of rest as before the traction was applied (fig. 21A). This paper possesses elasticity and an inherent tendency to return to a state of rest, but the phenomena are in a reverse order to that observed in the fibro-elastic mesh-work of the suspensorium uteri.

Figure 22A represents the fibro-elastic mesh-work filling the parametrial and parapelical space in a state of physiological tonus or rest (potential ligament). It is an open mesh-work,

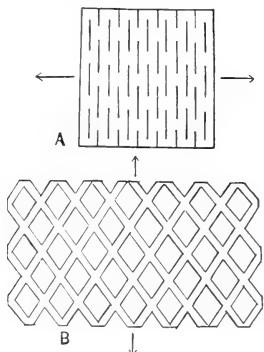


Fig. 21 A piece of tissue paper folded and cut (A) so as to form¹ a mesh work (B) that possesses elasticity and an inherent tendency to return to a state of rest as in A, but in a reversed order to that of the fibro-elastic mesh-work of the suspensorium uteri.

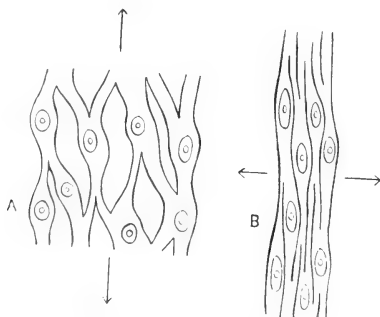


Fig. 22 Drawings illustrating the effect of traction upon fibro-elastic mesh-work. (A) Fibro-elastic mesh-work in state of physiological tonus or rest (potential ligament); (B) fibro-elastic mesh-work in state of traction (actual ligament).

the meshes of which are filled with areolar or adipose tissue. If traction is made as indicated by the arrows, the condition seen in figure 22B will result, the fasciculi and lamellae will be approximated and a transitory ligament will form. Remove the traction and by virtue of the inherent elasticity or resiliency the mesh-work returns. These simple experiments demonstrate the mechanism of the fibro-elastic suspensorium uteri.

The fibro-elastic network is like a net that has been symmetrically attached at a periphery and inserted into the two sides of a body, which is suspended by it. Traction downward on this body approximates the threads of the net which appear to diverge from the point where they are attached to it. If the body is released it is immediately carried upward until the inherent elasticity of the netting is satisfied, when a state of rest is established. The threads of the net no longer appear divergent at the sides of the body for they appear as a part of the net-work.

Such is the author's conception of the manner in which the uterus is suspended by the fibro-elastic tissue of the parametrial and parapical space and which is aided by the pelvic diaphragm and peritoneum. From what has already been said in this paper, the full import of the argument should be clear to all.

The normal workings of the suspensory mechanism is best observed in the virgin pelvis. Various factors begin to operate at the time of puberty, which may sooner or later weaken the power of the uterine or vaginal supports. Aside from the weakening effects of parturition, with possible injury to the pelvic floor, the pernicious effects of chronic constipation and tight lacing have to be taken into account. These slowly cause the fibro-elastic tissue to give away with loss of the normal resiliency or tone.

The writer agrees with Fothergill (15) in stating that prolapsus uteri may not occur even in long standing laceration of the perineum, providing the fibro-elastic suspensorium retains its tone. In the section on variation, the writer has pointed out that the relative size and strength of parts may vary greatly in the same and different individuals. A weak pelvic floor will

not bring about prolapsus uteri if there happens to be a well developed fibro-elastic suspensorium; on the other hand, a strong pelvic floor will delay a prolapse when a weak suspensorium is present. A lacerated perineum is always a source of danger. The applied facts should be clear.

With Cameron (13), the writer suggests that an attempt must be made to restore the weakened fibro-elastic support, if success in treatment of prolapse and mal-positions of the pelvic organs is to be obtained.

XI. CONCLUSIONS

The material studied for the preparation of this paper seems to justify the following deductions and problems:

The plicae sacro-uterinae or recto-uterinae are the homologues and analogues of the plicae recto-vaginales of quadrupeds.

The musculi recto-uterini of the higher Primates are the homologues and analogues of the same in quadrupeds.

The plicae recto-uterinae and their intimately associated musculi recto-uterini are primitive, appearing in the vertebrate series before the fibro-elastic suspensorium uteri or a well developed musculus levator uteri.

The suspensorium fibro-elasticum uteri has been gradually evolved with the assumption of the erect attitude in locomotion, and is not present, or is present only in a very rudimentary way in animals which habitually assume the horizontal attitude in locomotion. If present in a rudimentary or primitive form, it is only concerned in maintaining to a greater or less extent the cephalic portion of the vagina.

In woman, and the females of the higher Primates at least, the supports of the uterus are three in number, namely:

1. The suspensorium diaphragmaticum (pelvis).
2. The suspensorium peritoneale.
3. The suspensorium fibro-elasticum.

The latter being the chief and essential support, the others being accessory.

The suspensorium fibro-elasticum consists of a fibro-elastic network supporting vessels and nerves, and contains the poten-

tial ligaments of the uterus and vagina. The meshes of the network are filled with aerolar tissue which permit the fasciculi and lamellae to move freely over each other.

The potential ligaments are the *ligamentum transversale colli* of Mackenrodt, *ligamentum sacro-uterinum*, and the *ligamentum plico-vaginale*, which become actual through traction on the uterus and vaginal vault.

The three above-named ligaments are directly continuous with each other at the sides of the uterine cervix and vaginal vault and with the general fibro-elastic network to the periphery.

The fibro-elastic network in a state of physiological rest forms an open mesh-work and possesses an inherent tendency to return to a state of rest after traction on the uterus or vaginal vault has ceased to operate, and neutralizes or minimizes downward pressure through this same property.

The fibro-elastic network in a state of rest prevents compression of the venous and lymphatic vessels by preventing collapse of their walls. All traction upon it more or less compresses the vessels and it hence becomes an aid to the venous and lymphatic circulations.

The upward and downward movements of the *suspensorium diaphragmaticum* augments the action of the *suspensorium fibro-elasticum* in aiding the pelvic circulation, and in this way it is analogous to the thoracic diaphragm.

The *suspensorium fibro-elasticum* permits the uterus being depressed to the introitus vulvae. The return of the uterus to its normal position being first aided by the *diaphragmatic funnel*, and completed by the *suspensorium fibro-elasticum*.

The *musculi levatores uteri* are derived from the *muscularis uteri* and constitute a mechanism by which the uterus can automatically raise itself on the *suspensorium fibro-elasticum*. By its more or less rhythmical or intermittent contractions, in conjunction with the *muscularis uteri*, it aids the venous and lymphatic circulation in the respective peri-uterine plexuses.

The *plicae sacro-uterinae* or *recto-uterinae*, with the intimately associated *musculi recto-uterini*, lifts the uterine

cervix and vaginal vault upward and backward, through stimuli received from a distended upper rectum or from other sources.

Those fasciculi and lamellae of the fibro-elastic network that arise from the presacral fasciae and along a plica sacro-uterina, and which have a general trend to the vaginal fornix and uterine cervix, behind and below the point of attachment of the ligmentum transversale colli, constitute the ligamentum sacro-uterinum, potential or actual (transitory).

Those fasciculi and lamellae of the fibro-elastic network arising from the fasciae of the levator ani, obturator, or sheath of the hypogastric vessels in the lateral wall of the pelvis, and with a general trend toward, and attachment to the side of the uterine cervix below the uterine artery, and above the ligamentum sacro-uterinum, constitute the ligamentum transversale colli potential or actual (transitory).

Those fasciculi and smaller lamellae arising from the stratum fibrosum of the peritoneum of the anterior two-thirds of a plica sacro-uterinum or recto-uterinum, and inserting into the sides of the vaginal vault below the ligamentum sacro-uterinum constitute the ligamentum plico-vaginale, potential or actual (transitory).

Any weakening of the suspensorium diaphragmaticum, which will result in a falling of the pelvic floor, or when coupled with a lacerated perineum, will result sooner or later in an over-stretching, and a giving way of the suspensorium fibro-elasticum, and must result in a vaginal or uterine prolapse, or malposition of the uterus, with consequent disturbance of the fibro-elastic and muscular mechanisms, which will be rendered more or less inoperable, with resulting venous stasis and increased weight of the pelvic viscera.

In conclusion, the writer desires to acknowledge his indebtedness to Prof. A. W. Meyer, for advice, and to Prof. William Ophüls and Dr. Edgar D. Downing, for the permission to examine and use material from the autopsy room; to Prof. E. C. Dickson for the preparation of the photomicrographs.

The work was begun while the author was connected with the division of anatomy.

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SPOLIA ANATOMICA, ADDENDA II

ARTHUR WILLIAM MEYER

From the Division of Anatomy of the Stanford Medical School

TWENTY-TWO FIGURES

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A CONGENITAL INTRA-CRANIAL, INTRA-DURAL ADRENAL

The cadaver containing this strange anomaly was that of a male native of Germany. The cause of death at the age of 73 years was assigned to chronic cardiac disease. Fortunately for us the body had not been autopsied. It had been received three days after death and had been preserved as is customary with us for dissection.

Messrs. Kelker and Geistweit, two of our medical students, who removed the brain in the course of their dissection called my attention a few minutes after removal of the brain, to a small body attached to the spinal portion of the left accessory nerve.

This body which was roughly cylindrical in form lay upon and was attached to the nerve along its dorso-medial surface. It was located directly cranial to the place where the vertebral artery pierces the dura. The body and a portion of the nerve were excised by myself in the presence of these gentlemen.

Since I took the enlargement for a neurofibroma I did not note its vascular or other relations as I should have otherwise done. The specimen was carefully removed, however, and its remarkable preservation has surprised everyone who has examined sections microscopically. Yet the cadaver had not been embalmed until three days after death. Dissection was not begun until seven months later and the specimen not removed until nine months after receipt of the body.



Fig. 1 Intra-dural adrenal, external view. Natural size. *a*, pedicles; *b*, loose outer capsule.

The specimen which is roughly cylindrical and measures 1.5 by 0.8 cm. is represented in natural size in figure 1. Upon inspection it is evident that the corpus proprium is surrounded by a loose capsule which bulges here and there and very evidently contains blood and pigment. The stalks which leave either pole of the specimen look somewhat like blood vessels but feel rather firm and cord-like.

Upon transverse section at its midpoint, it is seen that a very symmetrical white cylindrical body with rounded ends is surrounded by a scarcely discernible capsule and contained in a very loose outer envelope. The latter contains some blood in its looser portion only, for it is closely apposed to and seems to fuse with the capsula propria in the other half of its perimeter. It is this outer capsule which gives the specimen the peculiar irregular outline seen in figure 1.

The consistency of the specimen as well as its color made one think of the cerebral cortex. Under low magnification the cross-

section is slightly oval in form. The main portion of the section is composed of a fairly uniformly-constituted, slightly-granular mass with radial striations as represented in figure 2. The connective tissue capsule which varies greatly in thickness is quite closely applied to the contained tissue. Over that half of the perimeter where the outer capsule is thinnest the outer portion of the latter is reflected and forms a thick folded cushion of connective tissue which contains fat and a mass of blood. In the space between this outer reflected portion of connective

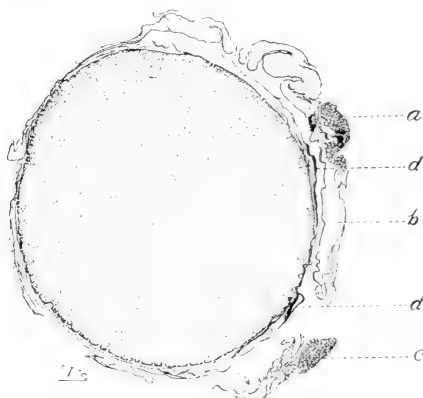


Fig. 2 Intra-dural adrenal. Cross sections under low magnification. *a*, fat; *b*, connective tissue; *c*, blood; *d*, muscle.

tissue and the inner portion of the capsula propria there is a large space which seems to contain some blood.

Under higher magnification the nature of the specimen at once becomes evident for very characteristic glomerular and fascicular zones can be recognized instantly. The former although very narrow, is very characteristic in many places. The latter is relatively longer than in human adrenals and shows a far better radial arrangement. In fact both these zones in this specimen remind one strongly of the appearance of cross-sections of the adrenal of the dog. The reticular zone is composed of a

solid mass of cells near the center of which an irregular circle of somewhat darker cells is seen as indicated by the shading in figure 2. Although these darker cells are not equally abundant in all sections of the portions cut serially, they remind one very strongly of cells from the medulla of the adrenal. The arrangement of the very evident fascicular zone is so symmetrical that the appearance of the section may justly be said to be pretty.

Under still higher magnification the cell boundaries and the nuclei are seen to be splendidly preserved in many areas as shown in figure 3. Since the walls of the capillaries are collapsed they are inconspicuous except here and there in the outer zone.

Only minor evidences of degeneration are present except in certain very small areas in the fascicular zone, which are composed of small masses of granules which stain deeply with hematoxylin. The central portion of the sections in some portions of the specimen, contains a few small clefts which do not look as though they resulted from post mortem changes or shrinkage. The relatively thin and loosely applied connective tissue capsule sends fine septa into the glomerular zone from its inner looser portion. Its outer looser portion also contains fat and unites with masses of extra-capsular connective tissue which contain fat, blood vessels and also some relatively large and many small masses of chromaffine cells. These irregularly-formed and irregularly-distributed cell masses stain deeply with chrome salts and in many places are arranged indistinctly curved cords. Since some space is left between these cords of cells the structure of the carotid gland is simulated quite closely in these areas, a small portion of which is shown in figure 4. Large and small very definitely circumscribed ovoid masses of chromaffine cells are also contained in the capsula propria. In these masses the cells are not so definitely arranged into cords however. One of these chromaffine bodies is so large that it has indented the glomerular zone beneath it as shown in figure 5. Chromaffine cell groups and fat are also found in the capsular extensions on both ends of the specimen, and on one end a great deal of pigment was found scattered about as fine gran-

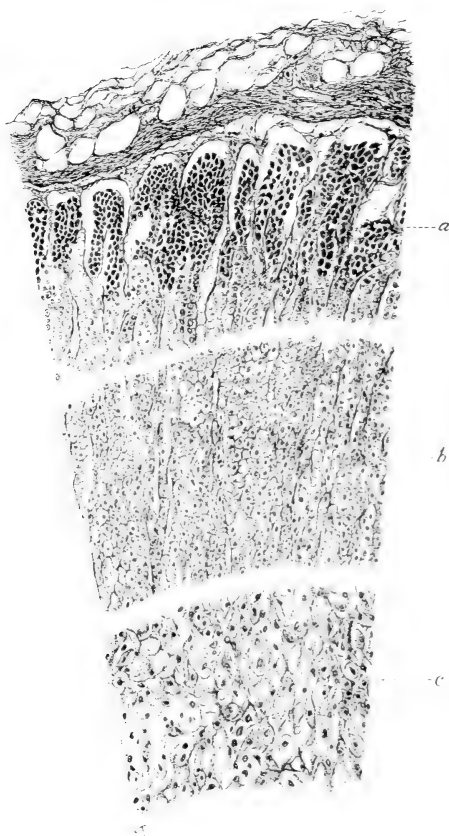


Fig. 3 Portions of the glomerular, fascicular and reticular zones and medulla.
× 475.

ules or as larger masses in the connective tissue which formed the extremity. It was this end which contained only a very small group of chromaffine cells in the portion examined in serial sections. Free erythrocytes were also found in this por-

tion which nevertheless was relatively non-vascular and the connective tissue of which was not so well-preserved. Considerable areas of degenerated blood were also contained in it besides a small oval body with a thin fibrous capsule, composed of granular material containing a small number of degenerated cells of various kinds, the identity or origin of which could not be determined.

But more surprising than all of these constituents is the presence of a good-sized bundle of striped muscles muscle in the external surface of the outer portion of the reduplicated capsule.

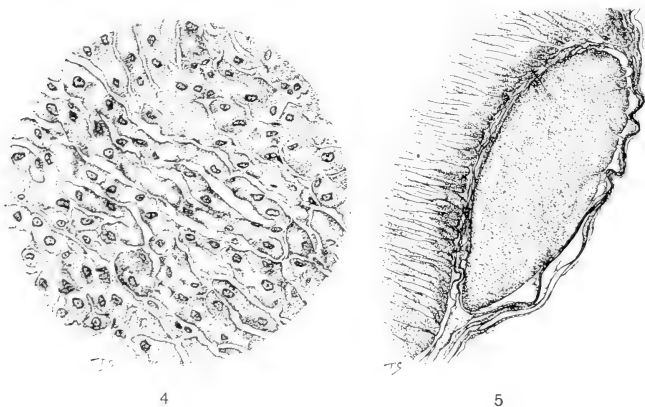


Fig. 4 Chromaffine cell masses from outer capsule. $\times 750$.

Fig. 5 Relation of large intra-capsular chromaffine body to the adrenal.

Until the direct connection with and indeed the inclusion in the reflexion of the capsula propria, of this muscle mass had been conclusively established by following the serial sections I was inclined to regard it as an accidental inclusion in spite of the suggestive staining reactions of the strand of connective tissue with which the portions first seen were so intimately associated. These muscle fibers are so well-preserved that the transverse striations are very plainly visible in some of them. Their location is indicated at *c* in figure 2. Strands of the spinal portions

of the accessory nerve are also contained in the capsule which also contains very wide exceedingly thin-walled vessels and a good deal of fat. It is very well-preserved except in certain small areas.

The structure of this specimen and of the surrounding tissues make it very evident that it is not metastatic in origin. Not the least indication of malignancy can be seen anywhere and the only possible explanation of its presence here, it seems to me, is that a small mass of early embryonic mesenchyme was in some way included in the dura. This mesenchyme must then have differentiated into all the constituents of the adrenal, into chromaffine bodies, striated muscle, connective tissue and fat, the presence of which tissues prompts one to put the specimen among teratomata.

Various writers on pathological anatomy seem to be agreed that the so-called primary hypernephromata occur only in the abdominal cavity. It is said that they are found but rarely in the liver; that they occur mainly in the kidneys but rarely also in the liver, ovary, testes, ureter and the broad ligaments of the uterus. Upon the advent of malignancy these aberrant adrenals may, to be sure, metastasize as any other malignant growth to any part of the body. None of the writers consulted have referred to or have themselves described anything at all comparable to what is here reported, however, and were it not for the fact that this supernumerary adrenal is accompanied by striated muscle one might, in spite of the chronological difficulties even, be inclined to consider the possibility of later transportation or even of the migration of specific cell masses which then formed the various complexes. But its location on the spinal portion of the accessory nerve between arachnoid and dura together with the peculiar vascular relations which it undoubtedly must have had with the meningeal vessels, alone make such a supposition quite untenable. Such a supposition would also imply the transportation of all these various cell masses at least in part by the cerebro-spinal fluid, to this peculiar location.

The very small amount of medulla within the specimen with restriction of the sympathetic elements almost wholly to the aberrant and extra and intra-capsular isolated chromaffine bodies is remarkable however, especially in that location. From the appearance of the specimen one gets the impression that although the sympathetic elements attempted to penetrate the cortical mass they only succeeded in reaching the capsule and the surrounding connective tissue leaving the corpus proprium overwhelmingly epithelioid.

THE RELATION OF SKELETAL TO BODY WEIGHT IN THE ADULT GUINEA PIG

From computations based on statistics given by Donaldson ('15) in table 53 the weight of the dried skeleton forms 1.94 per cent of the body weight in the new born, 4.38 per cent in the half grown and 4.09 per cent in the adult rat. Waldeyer ('10) gave the weight of the skeleton of two women of 40 years as 3.306 kg. and 3.585 kg. and that of a centenarian of 102 years as 1.185 kg. Although the body weights are not given, the weights given for these three fat-free skeletons indicate that they formed from 3 to 6 per cent of the normal average body weight. From the accompanying table it is seen that the skeleton of the adult guinea pig forms a somewhat smaller percentage of the body weight, than that of man but somewhat less than that of the rat. One would I think, expect this from a mere comparison of the body forms of the rat and guinea pig.

Since only ten guinea pigs were used for this determination the percentages obtained can, to be sure, not be regarded as being so near the actual for the guinea pig, as are those of Donaldson for the rat. Nevertheless with three exceptions, the percentages obtained agree very well indeed, thus reducing the probable error. Number 26 was not pregnant and hence less fat. Pig No. 35 was in the early and pig No. 20 in the late stages of pregnancy. Hence the divergences noted in these cases may probably be accounted for by these things. Similar differences are also found in Donaldson's large series and could, to be sure, be accounted for very easily by varying conditions of nutrition alone.

All animals in this series of ten pigs were pregnant, save No. 26. The weight of the uterine contents was, however, always subtracted from the total body weight before percentages were calculated.

The skeletons were cleaned by heating the fresh carcass in a 1 per cent solution of gold dust for five to seven hours. They were then dried for one week in a thermostat at a temperature of 54 to 55°C. after which the first weighing was done. Next they were placed in benzine for six to seven days in order to extract the fat and dried at room temperature for a week. The second weighing was then done. The treatment was exactly the same in several groups which were handled together.

From the percentages representing the relative weight before and after treatment with benzine it is seen that the reduction in weight amounts to 4 to 5 per cent of the weight of the cleaned, air-dried skeleton. This is a remarkably low amount of fat when compared with the results given for other animals.

NO. OF PIG	DURATION OF PREGNANCY	BODY WEIGHT	DRY SKELETON	PERCENTAGE	SKELETON DE- PRIVED OF FAT	PERCENTAGE
20	64	751.30	30.400	4.04	29.555	3.93
21	51	722.00	25.550	3.53	24.420	3.38
22	48	813.50	29.700	3.65	28.400	3.49
23	44	844.20	28.520	3.37	28.097	3.32
26	0	751.00	33.300	4.43	32.870	4.39
27	33	943.85	31.520	3.34	30.737	3.25
28	31	866.56	30.780	3.55	29.172	3.36
29	29	692.66	24.730	3.57	23.420	3.36
31	25	901.50	30.790	3.41	29.875	3.31
35	15	688.30	28.840	4.19	27.770	4.03

LYMPHOID NODULES IN THE LIVER OF *ALUCO PRATINCOLA*

In a specimen of the common barn owl—*Aluco pratincola*—lymphoid nodules of varying though small, size were found distributed at random throughout portions of the liver. The latter which looked wholly normal had been removed from a young owl about six months old. It contained no signs of inflammation or degeneration, either macro- or microscopically, and the young owl which had been under observation for some months had never shown any signs of illness.

These accumulations of lymphocytes mixed with erythrocytes were of microscopic size, the largest measuring only a small fraction of a millimeter. Most of them were irregular in form and included extensions of the parenchyma of the liver into them. Not rarely, however, one of the smaller follicles was surrounded by a very thin but distinct layer of connective tissue which could be regarded as a capsule. Those observed contained neither germinal centers nor an evident reticulum.

The lymphocytes were crowded together and included relatively few erythrocytes. When small collections of erythrocytes were present they were usually segregated fairly well from the lymphocytes. The nuclei of the latter were not pyknotic but vesicular, as is the case within the lymph follicles of lymph nodes. No polymorphonuclear leucocytes or giant cells were seen in these follicles and no evidence of any phagocytosis was observed. The lymphocytes were not arranged in cords but were scattered about miscellaneously and no lymph vessels or sinuses were observed. Comparatively large blood vessels were, however, not infrequently seen near these nodules, penetrating them, and forming sinusoids within them. The general appearance is indicated in figure 6.

LYMPHOID NODES IN STRIGIDAE

From an examination of 31 species including 14 families Jolly ('10) concluded that, among birds, lymph nodes are found only in some members of the group 'Lamellirostres' of the family Anatidae. Jolly failed to find them in *Branta bernicla* L. for example. Thirteen species of 'Lamellirostres,' all of which fall in the family Anatidae and one in the Phoenicopteridae were examined by Jolly. Ten in the former and one in the latter, viz., *Phoenicopterus roseus* Pall, contained lymph nodes. Of the other families examined none were found to possess lymph nodes. These families included: Alcidae, 1 specimen; Colymbidae, 2; Laridae, 1; Ardeidae, 2; Otididae, 1; Tetraonidae, 4; Columbidae, 1; Picidae, 1; Corvidae, 1; Alaudidae, 1; Sturnidae, 1; Fringilidae, 1; Strigidae, 1; Polyborinae, 1.

The inclusion of a specimen of *Strix flammea* L. in this list attracted my attention because I had incidentally in the course of other investigations, come upon what I took for lymph nodes, in a specimen of *Aluco pratincola* (*Strix pratincola*) the common barn owl. These nodes were found in the abdominal and thoracic cavities and were taken for lymph nodes when removed. Unfortunately, however, since they were incidentally removed and since two other owls were then available, the exact loca-

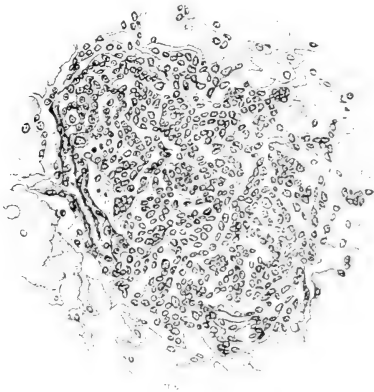


Fig. 6 Lymphoid nodule from the liver of *Aluco pratincola*. The surrounding hepatic parenchyma is merely indicated. $\times 475$.

tion of these nodes was not specially noted at the time of removal. The specimens were fixed in Zenker's solution, however, and were found to be unmistakably lymphoid in structure.

These nodes were cylindrical in form, 2 by 1 mm. in size and pale grey in color. This color may be accounted for by the fact stated by Jolly, that the sinuses of the lymph nodes of birds seldom contain enough blood to make the nodes look pink. Under low magnification no germinal centers or lymph sinuses were evident, although the parenchyma was an open one. In spite of the pale grey color of the gross specimens and the ab-

sence of blood-filled sinuses the stained mounted sections seen under low magnification suggested hemal nodes. Upon closer examination it was seen however that this was due to the presence of an unusually large number of large pink hyaline looking cells (fig. 7) which suggested erythrophages. These cells were scattered throughout the section of the node and contained vesicular nuclei which were generally located near the periphery. Although not contained in sinuses they are apparently somewhat comparable to those described by Jolly in the sinuses of lymph

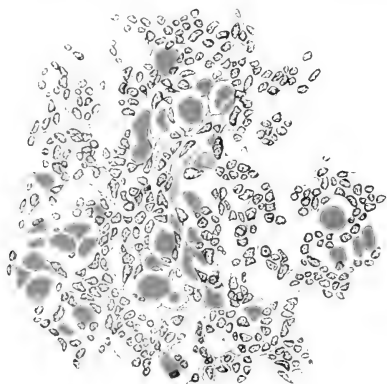


Fig. 7. Portion of a lymphoid nodule showing distribution of polykaryocytes and large acidophile cells. $\times 750$. *a*, capillary.

nodes of birds, as containing cellular débris within their protoplasm. The débris according to Jolly was composed of remnants of nuclei, erythrocytes and blood pigment all of which were "transformed into globular masses taking an acidophile stain."

Rarely polykaryocytes of the above type were also seen. These were extremely large and the protoplasm not infrequently contained what seemed to be remnants of nuclei. Sometimes larger, irregular masses which apparently had been formed by the coalescence of polykaryocytes were also found. These

masses were far larger than any I had ever encountered before in any lymphatic tissues examined (fig. 8). Since distinctly or indistinctly outlined erythrocytes were, however, never seen within these cells one is lead to doubt whether these large cells were really erythrophages for the nuclei of the erythrocytes unless destroyed before ingestion or extremely rapidly after that, should have been visible in some cells at least.

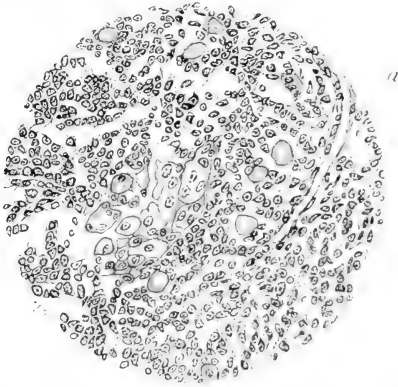


Fig. 8. Portion of a lymphoid nodule. $\times 515$. *a*, capillary.

CAPILLARY CAPSULES IN THE SPLEEN OF ALUCO PRATINCOLA

During the examination of sections of the spleen of the owl, under low magnification, small groups and isolated syncytial-like masses with a small central opening with a circular or more or less elongated form especially attracted attention. These capsules characteristic of birds gave the impression that the spleen was studded with extremely large multinucleated giant cells, were always contained in the areas of lymphocytes and were absent in those areas of the spleen which were largely or almost wholly composed of erythrocytes. Upon higher magnification it was found that the small central openings contained in these masses were fine vascular capillaries which were

surrounded by a capsule of an epithelioid syncytium which was from four to six times as thick as the caliber of the capillary. Whenever some of these capsules were cut more or less obliquely and also when adjacent capsules coalesced, large irregular masses with smooth outlines resulted, but whenever the capsules were cut transversely the small capillary was usually centrally located in the section. Not rarely two small capillaries one of

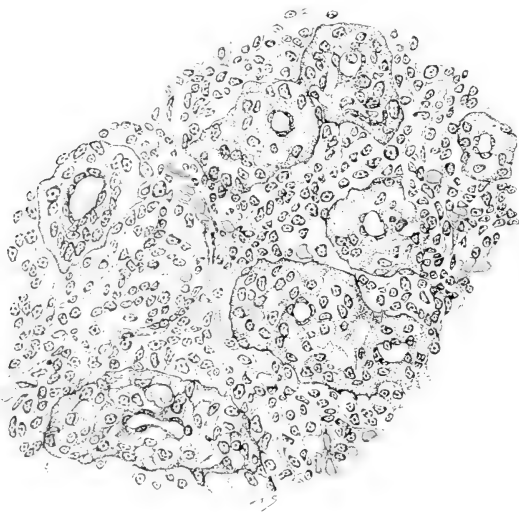


Fig. 9 A group of capillary capsules of the spleen of *Aluco pratincola*. $\times 750$.

which was eccentrically placed were contained in such a cross section. Some of these capsules lay directly beneath the splenic capsule or even caused it to bulge. They were always well-defined and sometimes surrounded the capillary at the point of branching. Because of this fact and because of the consequent fusion of such adjacent and other capsules and perhaps also for other reasons, the size of these capsules varied considerably although there was but little variation in the caliber of the enclosed capillaries (figs. 9 and 10).

Not infrequently two fairly concentric circles of nuclei were evident in cross section of these capsules. The outer circle lay directly beneath the periphery and the inner directly around the capillary. The latter was, of course, formed by the nuclei of the endothelial cells of the capillary and except for a slightly smaller size and a somewhat more oval form the nuclei forming the inner and outer circles seemed to be identical in appearance. They were always vesicular and contained a number of distinct chromatin granules. Sometimes they were distributed irregularly throughout the cross-section of the capsules. The inter-nuclear protoplasm stained pink with eosin and was non-granular. Cell boundaries were never recognizable and no cells except rarely a few isolated erythrocytes or fragments of such were seen in the syncytium of the capsules.

In some cases the capsules instead of being composed of an epithelioid syncytium were composed of such merely along a narrow margin of their periphery and in the region immediately surrounding the capillary. The intervening space is partly filled with a rarefied tissue the individual nuclei of which are surrounded by small more or less confluent, amounts of protoplasm giving these portions the appearance of mesenchyme.

Aside from these capsules the entire absence of Malpighian corpuscles attracted attention. Not a single corpuscle was found in the sections examined and the only substitute for them were these large circum-capillary capsules surrounded by lymphocytes. Since as many as a dozen of these capsules often lay quite closely together forming rather large pink-staining masses which were surrounded by blue-staining lymphocytes they were very conspicuous. The portions of the spleen examined contained few large sinuses, a considerable quantity of erythrocytes, few trabeculae and had a thin capsule.

Kyber ('70) found the capillary capsules in the dog to be 0.05 mm. wide and 0.15 mm. long. According to Kyber, Fenenko also described capillary capsules first so-named by Schweigger-Seidel, ('62) who worked on the pig. They were discovered by Billroth, ('57) in birds and described by Müller ('65) in frogs.

reptiles and birds. Bannwart ('93) found them in the cat and Kultschitzky ('95) in *Putorius vulgaris*.

Kyber thinks that they are formed by local distensions (Aufreibungen) of the adventitia of the arteries, which then enclose the splenic parenchyma in the form of a thin sheath of the ends of the terminal arteries. Kyber states that previously to his publication they had been described in the pig, dog, cat and hedgehog only, but Bannwart states that Müller found them indicated in the mole and rabbit also and found them in capsules but non-striated muscle and polymorphonuclear leucocytes were never noticed. The very large size of the capsules in the owl's spleen as well as the large caliber of the capillary are evident by a mere reference to the figures.

PHAGOCYTOSIS IN THE LIVER OF FELIS DOMESTICA

The animal from which this specimen was obtained was an old but well-nourished pregnant female. The four foetuses and one abnormal ovum were about 3 cm. long. Although the cat had been handled very carefully and was killed in a gas chamber the abdomen was found full of fresh blood. Upon inspection of the viscera it was found that blood was oozing from the whole of the ventral surface of the liver and upon gently wiping the surface with a wad of cotton it was evident that the oozing came from small dark discrete points, the central veins. A little firmer wiping abraded the thin capsule and exposed the parenchyma of the liver. The latter was very friable and yellow but the other viscera appeared macroscopically normal except that the ovaries were cystic.

Upon microscopic examination the capillaries of the liver which contained but little blood, were found to contain numerous erythrophages as shown in figure 11. These cells which had a distinct cell membrane and a flattened crescentic nucleus which had been pressed against the cell wall, were about the size of the cells of the hepatic parenchyma. They were engorged with erythrocytes the outlines of which were still plainly visible in many of them. No phagocytosis was present in the spleen, sections of which showed a rather rarefied parenchyma.

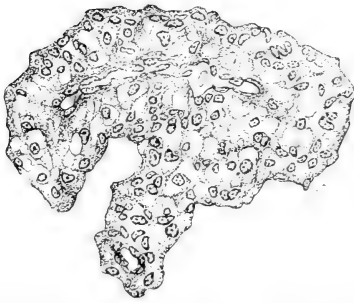


Fig. 10 Two adjacent partly fused capsules containing a branching capillary. $\times 720$.

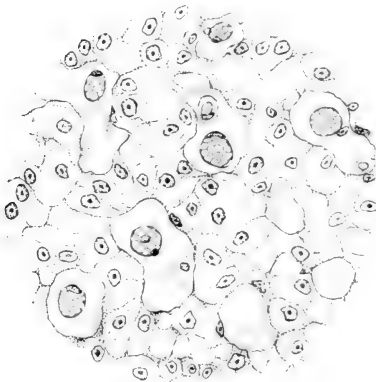


Fig. 11 Phagocytosis in the liver of a cat. $\times 1050$.

The kidneys showed a few old lesions but nothing else of consequence.

In spite of the very active phagocytosis of erythrocytes in the capillaries of the liver of this cat I found no indication whatever of phagocytic activity on the part of the hepatic parenchyma itself as observed by Browicz ('99). Nor did I see instances of

phagocytic activity on the part of the attached endothelial cells such as was observed by Heinz ('01). Nevertheless, the character of the phagocytic cells suggests an endothelial origin and to that extent confirms Kupffers ('99) conception of the phagocytic capacities of the endothelium of the liver. The entire absence of phagocytosis in the spleen of this cat would also seem to preclude an extra-hepatic origin of the erythrocytes in this case.

Hemorrhage into the abdominal cavity and apparently always from the liver, was also observed not infrequently in other animals killed by the use of illuminating gas. Since the animals are placed in a roomy lethal chamber and the gas turned on so slowly that death almost invariably results without a struggle, I can only suggest that for some reason unknown to me, extreme congestion of the liver with a possible change in permeability of the capillary and capsular walls must occur during death by the use of illuminating gas.

THE ARCHITECTURE OF THE PROXIMAL EXTREMITY OF THE HUMERUS

While scrutinizing the nature and the extent of the epiphyseal line in mature bones my attention was attracted to small areas adjacent to the epiphyseal line, in which the spongiosa is not infrequently absent. Often when not completely absent it is rarefied. These areas which were observed in the humerus, recalled Wards triangle and the similar rarefied areas in the spongiosa of the bodies of the vertebra and of the os calcis. Closer examination of a series of humeri showed that rarefaction or absence of the spongiosa were correlated with the retention of the epiphyseal line or plate. These absorption areas were always located in the lateral region of the shaft directly under the greater tuberosity a region in which the epiphyseal plate is best preserved. It is interesting and significant that a similar although not a corresponding absorption of the spongiosa can also be rarely seen near the epiphyseal line of the great trochanter of the femur.

As shown in figure 12 these absorption areas occur on both sides of the epiphyseal plate and their size and the completeness of the absorption of the spongiosa, seem to vary directly with the completeness and strength of the epiphyseal plates.

Whenever a part or the whole of the epiphyseal line was marked by a partial or complete bony septum or by two parallel thinner septa, the areas devoid of spongiosa were found the largest. Sometimes, however, there was only a partial absence or a rarefaction of the spongiosa and as shown in figure 12 when no epiphyseal line was evident there was no indication of absorption. If on the other hand the epiphyseal line was absent altogether no absorption areas were found. This relationship would seem to suggest that a strong epiphyseal plate relieves the spongiosa about it of most even if not of all, of the strains and stresses and thus causes its atrophy and rarefaction and finally its complete absorption. This conclusion would seem to be supported by the occurrence of all manner of transitions between a perfectly normal spongiosa and complete absorption and it is significant that the trabeculae of the spongiosa which are still preserved extend mainly at right angles to the epiphyseal plates thus acting as braces to relieve lateral strains.

That the presence of such remnants of the epiphyseal plates has resulted in the absorption of the spongiosa is also suggested by the specimen of the humerus shown in figure 13. This specimen shows a large absorption area in the spongiosa, directly beneath the site of the great tuberosity, in which the spongiosa has completely disappeared probably in consequence of the disuse following articular disease. This humerus came from an extremity in which the long head of the biceps and the compacta in the region of the tuberosities had been completely destroyed by arthritis. There is no evidence of pathological processes within the spongiosa, however. Although the compacta of the humeral head shows some erosion it is also very evidently atrophic. Since the spongiosa around the empty area shows nothing suggestive of a pathological process it seems not unlikely that in this case absorption of the spongiosa may at least have been hastened by, even if not directly caused by, a lessened use during disease.

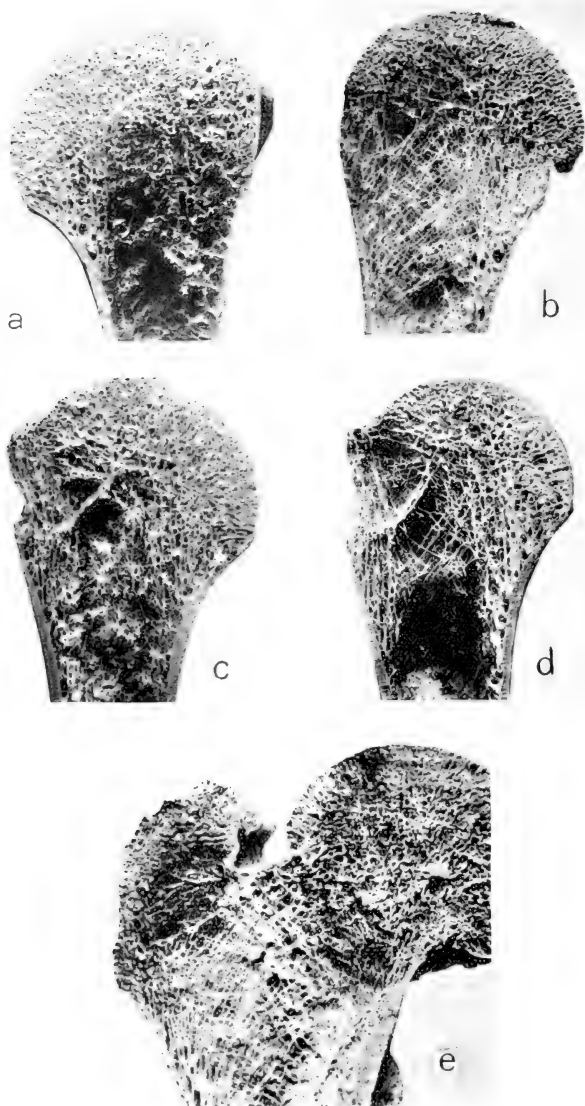


Fig. 12 Absorption near epiphyseal plates in three humeri and a great trochanter of the femur.

SERIAL TRANSVERSE BONY MEDULLARY SEPTA OF THE TIBIA

von Recklinhausen ('93) described transverse partitions as not infrequently occurring in the broad and thick portions of the diaphysis of long bones where the compacta is thin. He states that as many as twelve such partitions may be present in the lower end of the femur when the latter seems inflated (*aufgetrieben*) when viewed from the outside. According to von Recklinghausen transverse septa may also occur anywhere in the tibia and rarely also in the fibula and ulna, but never in the



Fig. 13 Absorption area under the greater tuberosity in a case of chronic articular disease.

humerus and in the upper femoral regions. They were also found regularly in the bones mentioned when multiple exostoses were present, in femora with a short neck and, in general, in bones of light weight possessing the loose texture characteristic of osteomalacia. They were also found in bones from females below ten to twenty years and also in the upper extremity of the tibia of a twelve year old dog. von Recklinghausen emphasized that these septa may be distributed at regular intervals and may be found even in the middle of the shaft where the compacta is thickest. He concluded that they are remnants of the solid

portions of the bone which originally formed the epiphyseal plates of the growing bone, and have no mechanical significance.

The occurrence of isolated complete, partial or fenestrated transverse septa in the shafts of certain long bones, is of course very common, but the presence of a series of parallel septa at comparatively short intervals as shown in figure 14 is rare.



Fig. 14 Tibia with transverse septa.

This specimen also deserves special comment because of the rarefaction and the nature of the spongiosa between the septa. The atrophy of the spongiosa near and between the septa can, it seems to me, be attributed to the presence of the transverse septa. Although none of these five partitions are complete and although all of them are fenestrated, rarefaction of the spongiosa is especially evident near them. All of these septa are found in the dorsal portion of the proximal extremity

of the tibia and three of the five are much stronger than the other two which are composed merely of a framework of spongiosa. The spongiosa between the septa is represented by a few very fine strands only and these extend mainly in a ventral direction at right angles to the septa.

Nothing observed in this specimen militates against von Recklinghausen's belief that such septa are remnants of former epiphyseal plates, but transverse septa located at or very near the midpoint of the shaft of a long bone could hardly be regarded as having such an origin. Moreover, the rarefied spongiosa in the proximity of the septa and also between them, indicates quite clearly that such septa are not necessarily or even very probably, wholly without mechanical significance as von Recklinghausen suggested.

INIAL FOSSAE AND CANALS

The skull shown in figure 15 I owe to the generosity of one of our former students Mr. Benjamin R. Hewitt. It was taken from an Indian Mound near San Jose, California, and as shown is markedly deformed. Although the deformation is marked it is nevertheless quite symmetrical and shows itself mainly by a decided flattening in the occipital region and of the vertex. The forehead is an extremely receding one, a gentle depression marks the glabella and the supra-supraciliary regions. The skull is decidedly prognathous and the norma frontalis which forms an angle of approximately 40 degrees with the vertical, is roughly parallel to the occiput. The obelion is located about 3 cm. posterior to the line passing through the mastoid processes and is marked by two small pits about 3 cm. apart which lie on opposite sides of the sagittal suture and which probably represent obliterated emissary foramina. The cerebellar fossae are deep, that on the right being the deeper, as usual. The floor of the left fossa is exceedingly thin and defective, partly no doubt from post mortem decay. The portion of the occipital bone bounding this fossa is much thinner, however, being only a few millimeters thick. All the sutures are still evident on the exterior and a good-sized 'os Incae' is present. The linea nucha



Fig. 15 Indian skull with inal canal *a*, rear; *b*, side view

suprema is very evident but the linea nucha superior can not be identified definitely. A very marked external occipital protuberance is present. This protuberance takes the form of a

torus 4.5 cm. long and somewhat over 1 cm. high at its midpoint. One centimeter above the protuberance there is found a very marked but definitely circumscribed oval pit 1.2 by 0.7 cm. deep. The canal leading from this pit is obstructed in part, by a plate of bone about 1 mm. thick the edge of which has very probably been destroyed.

On sagittal section of the skull it is seen that the canal is somewhat irregular in form being obstructed by the thin plate of bone mentioned above, on the inferior portion near its inner orifice. The latter is irregular in form and measures 7 by 7.5 mm. Since the canal is funnel shaped the outer orifice is much larger, measuring 1.8 by 0.8 cm. in the transverse and vertical diameters respectively.

The mastoid foramina are small, the parietal are obliterated but the jugular foramina are large. Hence it seems to me that one could hardly assume the presence of obstruction to the venous return and regard this canal as an enlarged occipital emissary vein. Moreover, were it to be regarded as such it would for several reasons be an extremely rare instance. The occipital emissary vein is usually small, it not infrequently pierces only one table and is often absent altogether. It is true that the canal in this skull leads partly into the *sulcus* for the left lateral sinus but the character of the canal itself is wholly different from the enlarged mastoid canals not infrequently seen especially in rachitic skulls as emphasized by Merkel. It is possible, to be sure, that the canal in this skull has nothing in common as to its origin, with the *sulci* and *fossae* found in this region in the other skulls. Nevertheless if it is to be regarded as an enlarged emissary canal its character can only be explained by assuming a decidedly deforming influence upon it by the forces which deformed the skull.

It is impossible to find a satisfactory embryological explanation for this peculiarity and since three other Indian skulls in a small collection of 60 possess, roughly similar depressions in exactly the same location, it is probable that these pits or defects have another origin. It is true that these defects lie in the region of the union of the interparietals with the supraoccipitals

but their character does not suggest a developmental origin. In one of the other three specimens there is a small circular depression about 3 mm. deep at the center and $1\frac{1}{2}$ cm. wide. The other two skulls merely have very irregular small depressions and a fourth shown in figure 16 has a definite larger depression directly in front of the superior nuchal line.¹

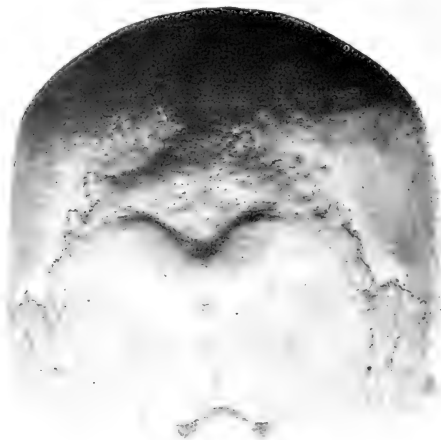


Fig. 16 Indian skull with peculiar depression

It is true that Frasseto ('02) in a purely theoretical and hypothetical discussion says that an inial fontanelle was described by Maggi ('00) and by Staurengi ('99). I have examined a number of papers by these authors but have been unable to find anything in their comparative anatomical studies at all comparable to what is here described and pictured.

¹ In view of Dr. Hrdlička's large acquaintance with skeletal remains gathered in widely different parts of the world and especially with American Indian remains, I brought the accompanying illustration to his attention. My expectations were fully justified, for Dr. Hrdlička has a series of specimens with similar, even if not identical, characteristics in the Smithsonian Collection.

NOTES ON 'SENILE' ATROPHY OF THE CALVARIUM

The erratic nature of bone resorption on the calvarium in the region of the parietals and sutures must continue to impress everyone. This is particularly true since we are in the habit of attributing the differences in relative degrees of senile atrophy between the bones of the upper and lower extremities, to differences in activity. Waldeyer ('10) also had recourse to such an explanation in connection with the findings in his unique study of the skeleton of a centenarian but in the case of the peculiar concentric atrophy of the calvarium we are left without this explanation.

Voigtel (1804), Lobstein ('24), Rokitsky ('44), Virchow ('54) and Maier ('54) were among the earliest investigators who described examples of this peculiar form of atrophy. Voigtel who refers to several earlier authors pictured a specimen from Meckels collections in which the atrophic area measured 3 by 2 inches. In one of the two cases reported by Virchow the bone in the atrophic area which measured 2.5 by 1.5 inches was, only " $\frac{1}{2}$ a line thick." According to Virchow the atrophy in the regions of the tubera parietalia never extends beyond the 'linea semicircularis' the insertion of the temporal muscle always definitely limiting the area. Virchow found atrophy present in other regions of the calvarium, however, and also noted joint changes.

Maier who likewise described two specimens of calvaria reported a case of death following fracture in one individual. Maier like Virchow, emphasized the porosity of the whole calvarium, the whiteness of the atrophic and the yellow color of the preserved areas, and spoke of the presence of a peculiar reticulated appearance due to the presence of lighter stripes among the yellow. Maier, however, found that the atrophic area extended beyond the 'lineae semicircularis.' In the first skull reported by Maier the bone in the region of the 'tubera parietalia' was translucent over an area as large as a 'Zwölfkreutzerstück;' that is, about 3.5 cm.; and as thin as 'Postpapier.' In the second skull the atrophic area was two inches long and one inch wide.

In reporting the case of a woman of 90 years who had suffered a fracture of the calvarium indirectly as a result of such atrophy, Humphrys ('90) also stated that "The most common parts for the extreme thinning are the parietal bones on the side of the sagittal suture, midway between it and the tubera, causing the remarkable symmetrical depressions of which many specimens exist." Humphrys also was impressed by these "changes of an opposite nature" in old age—the absorption from without and the deposit from within.

Rokitansky suggested a probable relation to lues but Virchow, Maier, Humphrys, Ziegler, Aschoff and others all refer the atrophy to senility alone. Smith ('06) called attention to the fact that this form of atrophy is rare in European crania and stated that Humphrys found only six instances of it in European museums. According to Smith this peculiar form of atrophy is common in ancient Egyptians and never affects the parts of the calvarium covered by muscles. Smith further stated that a ring of bone 1 cm. in diameter is always left around the parietal foramina. Although out of the 70 specimens examined by him, not one was found below the age of 25 or 30 years, Smith nevertheless concluded that "It cannot be regarded as a senile change because it frequently occurs in crania where the coronal, sagittal and lambdoid sutures show no trace of closing." Although this atrophy was not found limited by sex, Smith found it present only in skulls taken from the tombs of the wealthy from the period between the fourth and nineteenth dynasties. Smith came to the conclusion that this atrophy is not congenital but is due to a continuous, slight pressure because he found, "This cranial thinning only in those people who were accustomed to wear wigs of enormous proportions and of great weight." Smith added by the way of qualification, however, that a causal relation does not necessarily exist between the two.

The first calvarium upon which I wish to comment is one with a roughly rectangular depression 3 by 5 cm. long and 2 mm. deep over the mid-frontal region. The borders of this depression are very regular and smooth and a roughly corresponding

bulging of the inner table is present but the two do not coincide exactly and cannot definitely be attributed to fracture. The portions of the coronal and practically all of the sagittal and lambdoid sutures which show on this calvarium are obliterated internally but are still evident externally.

The thickness of the calvarium varies from 4 to 9 mm. and measures 5 to 7 mm. over the depressed area. The sulci of the middle meningeal artery are not deeper than usual but the arachnoidal and lacunar depressions are unusually large and deep, some of them extending well through the outer table. Yet on the whole this calvarium is heavy and its general appearance does not suggest senility. Compared with the measurements of Anderson ('00) which it is unfortunately very difficult to utilize because they are recorded in sixty-fourths (!) of an inch, this calvarium is above the average weight. The diploe are quite well-preserved but the lamina are thick. Only the right parietal foramen is preserved.

The second specimen which is very evidently senile has a small absorption area over the sagittal suture about 2 to 3 cm. anterior to the obelion, and a similar though less pronounced area on the lateral mid-parietal regions directly medial to the temporal ridges. The coronal and lambdoid sutures show faintly on the exterior and the whole anterior vault of the skull up to the absorption area in the mid-line shows definite vascular and nerve markings. The sulci for the right supraorbital nerve extend beyond the coronal suture.

The internal surface of this calvarium is rough and shows considerable deposit. There are several exostoses in the frontal region and also deep tortuous arterial sulci. The condition of the rest of the skeleton would suggest that a marked reaction probably of syphilitic origin, was present. Although the frontal sinuses are small this calvarium measures over 1 cm. in thickness. The lamina externa and interna are very thin but the diploe thick and well preserved. The coronal and lambdoid sutures are faintly indicated externally and the right parietal foramen is well-preserved.

The third specimen is a very light calvarium in which the location of the sutures is marked by sulci. There is a very shallow and narrow sulcus over the coronal suture but the sulci over the dorsal half of the sagittal and the lambdoid sutures are deep and wide and by their nature remind one at once of the peculiar large absorption areas in the parietal bones above referred to. As shown very imperfectly by the photograph in figure 17 there are roughly-corresponding, comparatively large absorption areas in the posterior mid-parietal regions. Both



Fig. 17 Calvarium showing absorption areas

these parietal absorption areas and the central sagittal area contain small areas varying from 1 to 4 sq. cm. in which the bone is less than 0.5 mm. thick. This calvarium which is light and thin, measures 7 mm. in thickness at the lateral border of the parietal absorption areas. This is the greatest thickness found. Except for a slight roughening along the superior sagittal sulcus there is no evidence of bone deposition on its anterior. The figure formed by the sagittal and lambdoid absorption areas although T-shaped, has nothing in common with the T-scars of the dolmen skulls reported by Manouvrier ('04). Since the latter have a mechanical origin being according to Obermeier,

due to scraping for aesthetic or dedicatory purposes they have a wholly different character. The slightly pitted outer surface of this calvarium also suggests senility. The sutures and parietal foramina were totally obliterated.

The fourth and most interesting specimen is from the body of a female 72 years old. The whole cadaver except the lower portion of the face, suggested senility and considerable loss of



Fig. 18 Deep 'senile' excavations in the parietals with the accompanying brain.

weight relatively shortly before death. The deep parietal excavations shown in figure 18 attracted attention as soon as the body was unwrapped and recalled the descriptions of Humphry, Maier and Smith and an illustration in Ziegler ('02). Comparatively slight sagittal pressure on either side in these areas, produced the peculiar crackling sound of thin bone.

The scalp was not abnormally adherent anywhere but the calvarium was apparently very thin except perhaps along the

ridges which surrounded the depressions. The large frontal sinuses were outlined indistinctly through the thin lamina externa. Upon removing the calvarium the dura was found decidedly adherent to the calvarium as is customary in old skulls. It was especially adherent over part of an area 2 by 1 cm. where the inner table of the right frontal sinus was completely absent. The dura here fused with the sinusal lining which was not especially adherent to the bony wall. There was no evidence on this calvarium of bone formation, except in the region opposite the left pterion where a small rough hemispherical nodule 7.5 mm. at the base and 3.5 mm. high projected into the cranial cavity. This nodule which was imbedded in the posterior end of the mid-frontal gyrus, was somewhat adherent with its base, to an oval depression to a rough plate of bone about 6 by 10 mm. in size on the inner table. The rough scale-like character of the inner table in the region of the frontal sinuses, a few scattered much smaller plaques of bone on the inner table and the deep arterial sulci with steep walls all suggested the deposition of bone on the interior.

As indicated by the depth of the fossae the absorption of the parietals is almost complete on both sides, their thinnest portions measuring less than 0.5 mm. over several small areas and less than 1 mm. over the whole of the rest of the floor of the depression which covered oval areas about 4 by 3 cm. These areas as measured on the level of the outer table were approximately 8 by 5 cm. large and were surrounded by a slanting wall about 1 cm. high. Although the thickness of these surrounding bounding walls on both sides, measured from 9 to 12 mm. the rest of the calvarium in the non-depressed areas was only 7 to 10 mm. thick. In addition to the marked absorption in the areas mentioned, that in the temporal regions was also very noticeable, yet this calvarium still measured 1.6 mm. in thickness, at a point 2.5 cm. above the internal occipital protuberance. About 3 cm. above the external auditory meatus it measured 3.8 mm. Although the thickness of the calvarium 1.7 cm. above the orbits was 1.7 cm., the inner table which was

absent in some places opposite the frontal sinuses was only 0.75 mm. thick and the outer only 1.25 mm.

Numerous small areas of total absorption were also present over comparatively large areas of the tegmen tympani. The three largest of these areas in the tegmen measured 3 by 2 mm. and while those on the right side were smaller the tegmen nevertheless looked honey-combed. Small absorption areas extend anteriorly quite close to the sulcus for the middle meningeal artery. The digital impressions and juga cerebraalia were not very evident.

The brain showed no very marked atrophy but the arachnoid was very thick especially over the whole frontal region. It was web-like and not unlike loose cotton in gross structure, about 4 mm. thick and very adherent throughout. After fixation in formaline the brain weighed 1035 gm. The stature of the body was 158.3 cm.

The evidences of senility were not confined to the skull, however, for the ribs were represented by a mere shell of very thin, pliable bone which could easily be compressed between the fingers. The costal cartilages except the first, however, showed no signs of calcification even in their interior. They contained only a few small grayish dots which had not, however, proceeded to the stage of calcification. The laryngeal and nasal cartilages were not calcified either and the mandible was not markedly senile. The preservation of the latter was due to the fact that portions of the lower incisors, the canines, the left and right lower premolars, the roots of the first right lower molar and the stumps of two roots of the second right lower molar were preserved. That the roots of the absent teeth had not been lost very long before death is shown by the irregularities of the gums due to unabsorbed remnants of the alveolar processes. The rest of the skeleton is markedly senile.

I have also been repeatedly impressed by the character of a general absorption occasionally seen in the posterior region of the parietals. Not rarely this absorption stops almost completely when the lambdoid suture is reached thus causing the squama of the occipital to rise like a wall along the suture. It

is exceedingly difficult to understand what can be responsible for the character of the absorption in this region of the parietals. The lambdoid suture appeared normal in all these cases and we cannot have recourse to muscle action as preservative agents. There was no evidence of rickets in these cases.

Although the senile character of such atrophies of the calvarium as here reported has been and may be called in question, yet in these cases the atrophy probably was not due to the continuous pressure of a lesser weight, as Smith concluded for Egyptian skulls. Although the ages vary considerably the histories of these cases unfortunately are not available. The fourth case was that of a woman born in this country. Hence weight bearing with the head, can be quite confidently excluded. Moreover, the erratic location of these atrophies as well as their peculiar shape makes it exceedingly difficult to conceive how pressure could be exerted upon those areas unless the head were born well-flexed upon the chest during exertion which would interfere seriously with respiration. Besides weight-bearing or pressure of considerable magnitude and for long periods of time do not result in such atrophy.

THE RELATION OF BRAIN ATROPHY TO CRANIAL ATROPHY

A case of very marked brain atrophy in an old woman, accompanied by congenital porencephally might be expected to furnish some evidence even if slight, of the relation of cerebral atrophy to the thickness of the overlying calvarium. Since the decrease in the volume of the cerebral cortex and the accompanying enlargement of the lateral ventricles do not cause a decrease in intra-cranial pressure it might be assumed that no deposit of bone should occur on the internal surface of the calvarium in consequence of brain recession. This is especially true since in cases of hydrocephalus an increase in intracranial pressure is accompanied by thinning of the calvarium and because constant pressure from other causes has a similar effect. Nevertheless, thickening on the interior of the calvarium in the lateral frontal regions in consequence of deposit of bone from within as

noted by Humphry, ('58) is very common in connection with atrophy of the frontal lobes in senility. But until more is known about the cause of deposition of bone on the inner surface of the calvarium it would be wrong to assume that there is a causal relationship between the two. Especially since we do not know why in one case of atrophy there is marked absorption within with consequent thinning of the calvarium and decided broadening of the arterial sulci with their final obliteration through absorption, while in the other, on the contrary, there is just as marked a thickening in consequence of deposition within accompanied by deepening of the arterial sulci to such an extent that large portions of the vessels may be almost enclosed.



Fig. 19 Primary congenital porencephaly

The brain showed in figure 19 was taken from the body of a woman 75 years old, dead of myocarditis. Neither the skull as a whole nor the calvarium showed any markedly senile changes. After fixation in formaline the brain weighed 1034 grams. However, there had been some post mortem drying and shrinkage. The meninges were normal but all the cerebral arteries were somewhat sclerotic. Atrophy of the left hemisphere seems clearly more marked than that of the right. This is evident even along the longitudinal fissure, the frontal lobe and the lateral and central sulci. Marked asymmetry in the shape and the arrangement of the gyri also exists. This is especially notice-

able in the porencephalic area which lies in the posterior extremity of the medial frontal gyrus and affects the latter and also the pre-central gyrus in the region around the posterior extremity of the inferior frontal sulcus. The defective area measures 2.9 by 2.1 cm. and is approximately 2 cm. deep. The nature of the surrounding gyri as well as the condition of the corresponding area on the right side and that of the brain as a whole seem to indicate that this defect is not due to senile atrophy but to faulty development. This assumption was confirmed by section of the brain. There were no evidences whatever of lesions and the gray substance was as thick at the bottom of the porencephalic area as elsewhere. It was merely a case of failure of this area to develop properly and the defect is hence truly congenital.

The peculiar arrangement and form of other gyri also suggests this. The arachnoid bridged over this depressed area and the overlying calvarium showed a gently rounded eminence on the interior and also a corresponding depression on the exterior. The calvarium here was also slightly thicker than the corresponding area on the other side but because of the corresponding depression of the outer table this increase in thickness was only very slight. The respective thicknesses at corresponding points on the two sides of the calvarium were 8 and 6 mm. The fact that the outer table was depressed over this area also indicates, it seems to me, that the local cerebral deficiency was a congenital rather than an acquired defect for, with the latter, one might expect a thickening of the calvarium from within unaccompanied by a depression from without.

Although the left hemisphere plainly looked more atrophic than the right it nevertheless weighed 11 grams more. The measurements for length, breadth and width of the two hemispheres were practically equal, but considerable differences between the volume of the two ventricles was found to exist. The volume of the right ventricle was 24 cc. and that of the left 20 cc. which accounts in part for the deceptive appearances. The volume of these ventricles is also considerably above the average found by Harvey ('09) who gave the ventricular volume for

brains with an average weight of 1314 grams as 15.08 cc. for the left and 13.49 cc. for the right side. Considerable differences in ventricular volume were encountered, however, by Harvey.

The character of this calvarium does itself not suggest senility. The parietal foramina are obliterated but the lambdoid suture is still preserved within, and the coronal sagittal and lambdoid sutures, especially the last two, are still well-marked externally. The whole inner surface of the calvarium is slightly rough, however, plaques of new bone are found here and there and the region of the superior sagittal sinus is marked by a broad ridge which was apparently moulded upon the broadened longitudinal fissure. No absorption areas are present externally and the arterial sulci are not especially marked. Indeed, those over the depressed portion of the calvarium which covered the porencephalic area are especially shallow thus also suggesting that there has been no special deposit of bone there. The thickness of the calvarium in the lateral frontal region is 1.1 cm. The diploe are sclerotic and the calvarium which has a thickness of 5 mm. in only a few places, is heavy and strong.

The clinical history of this case could not be obtained but a careful examination of the entire body revealed no evidence of defective muscular development or of asymmetrical atrophy as a result of developmental conditions or of paralysis. Section of the cord showed it to be symmetrically formed and no gross signs of degeneration could be detected.

The greatly emaciated condition of the cadaver was rather puzzling and remained unexplained until the stomach was opened. It was somewhat dilated but otherwise normal save for the presence of a papilloma directly in front of the pyloric orifice. This tumor which was 3 cm. long arose from an enlarged base which lay partly upon the gastric margin of the pyloric sphincter but which was not adherent to the gastric musculature. The body was 1 cm. wide and 0.5 cm. thick being flattened dorso-ventrally and the free portion distinctly papillomatous.

The pyloric musculature was not hypertrophied and the base of the tumor was alone sufficient to completely obstruct the ori-

fice. Since the papilloma was freely movable its inclination toward the antrum probably did not prevent it from being forced against the pylorus and thus further obstructing the passage of gastric contents into the duodenum. Hence it seems highly probable that this woman suffered much from gastric trouble and that her emaciation was largely due to the mechanical difficulty caused by the tumor.

THE CAUSE OF SOME LARGE PARIETAL EMINENCES

It is usually stated that large parietal eminences are due to a local displacement outward of both laminae of the skull. In the great majority of cases this explanation holds but in case of

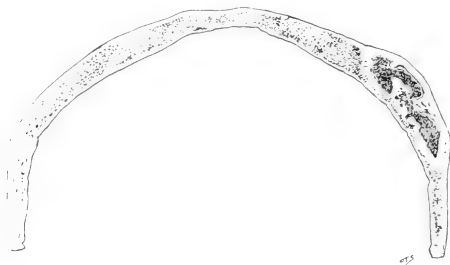


Fig. 20 Outline of a cross section of calvarium with a large parietal eminence

the calvarium outlined in figure 20 the large parietal eminence on the right plainly has a different origin. As the figure shows, the inner table of this skull has suffered no outward displacement whatever and the prominent eminence is due to a considerable thickening and a gradual deflection of the outer table. Sclerosis of the diploe is present and several areas of dense connective tissue and what looked like red marrow, are interpolated between the two tables the space between which is largely filled in by compact bone.

This calvarium which was taken from the body of a man 70 years old at time of death, is heavy partly because sclerosis is so complete in the frontal region and also because the calvarium

as a whole shows only slight evidence of absorption. Although the arterial sulci are only slightly deeper than normal, very evident thickening in the interior in the lateral frontal region is nevertheless present.

The greatest thickness of the right parietal eminence is 15.5 mm. and the thickness of the corresponding point in the left is 8.5 mm. The greatest thickness in the left lateral frontal region is 1.0 cm. and 0.9 cm. on the right. The thinnest point in this calvarium is in the right temporal region where the section measures only 2.5 mm. yet in spite of the thinning in this region the whole calvarium does not even remotely suggest senility. It is true that all the sutures are well obliterated and that the sagittal suture is marked by a very slight sulcus which extends over the metopic suture which can be recognized still. These things are, however, of but little moment. The right parietal foramen is still evident and there is no indication of concentric atrophy in that region.

The periosteum was not especially adherent over this bosse which measured 6 by 4 cm. in the sagittal and parietal planes respectively. Its surface is very smooth and it looks denser; that is whiter; than the rest of the calvarium but there is no good evidence of the presence of a pathological process and the rest of the skeleton was wholly normal.

EXTENDED FUSION OF THE SECOND AND THIRD RIBS

Although more or less extensive bifurcation of ribs is relatively common fusion is much rarer. In fact Bland-Sutton ('99) stated that fusion of the ribs resulting in the formation of bicapital ribs occurs only in connection with the cervical ribs or in relation to the first and second ribs. Disse ('96) made a similar statement. Dwight ('07) however, held that a bicapital rib may occur also by the fusion of the first thoracic with the second beyond the tubercles. Merkel ('99) in a fine summary, merely states that anomalies occur at the posterior ends in the region of the tubercles where the ribs may send processes toward each other and a similar statement is made by Nicolas ('11)

and Thompson ('14). In Rauber-Kopsch ('11) it is stated that now and then it is observed that the margins of two or more ribs may fuse for a greater or lesser distance. This statement was also made by Thompson ('13) who merely said that fusion of adjacent ribs may occur. In the second edition Thomson referred to Meckel.

Campbell ('69) reported a remarkable case of union of a number of ribs by cartilage and also by bone accompanied, however, by multiple exostoses and also by the formation of bone in the diaphragm and of cartilage in the right lung. The presence of so many exostoses and the occurrence of bone formation in the soft tissues makes it probable, however, that, in this case, the union of the ribs was due to a pathological rather than a developmental process.

Turner ('70-'71) referred to a case of fusion of the first and second ribs reported by Hunauld in 1740. Hunauld is also said to have possessed a fetal skeleton from the seventh month in which the upper five left ribs were united posteriorly and in which the sixth and seventh ribs were also partially united. In this article Turner pictured two specimens of fused ribs which from comparative anatomical considerations, he apparently declared to be fused cervical and first ribs. In a later article Turner ('82-'83) concluded that the two specimens in question were fused first and second ribs.

A fuller account than that contained in any of the above texts and handbooks is that given by Lane ('83). Lane gave several instances of fusion of a cervical with a first rib and of fusion of the first and second ribs. He found the common shaft formed by the union of the first and second ribs in one of his cases, $1\frac{1}{8}$ inches broad dorsally with a maximum width of $2\frac{1}{4}$ inches. The latter point was located $1\frac{1}{4}$ inches behind the termination of the lower rib. Unfortunately Lane did not give the length of the union but judging from his drawing this was as long as the breadth of the united ribs or $2\frac{1}{4}$ inches. Although the ribs in this specimen were said to be firmly united the "outer surface of the shafts still remained very prominent after fusion, being separated from one another by a deep groove. The inner surface of the com-

mon shaft was smooth, however, and presented no irregularity or ridge of any sort." Lane remarked that this specimen is peculiar in its great breadth and in the thinness and incurvation of its lower part.

Two similar cases were previously described by Turner, and Bryce ('15) stated that Valenti ('03) also described a case of fusion of the second and third vertebra accompanied by 'apparent' fusion of the second and third ribs for the greater part of their length.

The most comprehensive study of the occurrence of variations in ribs is that by Hrdlicka ('00) who examined over 1600 ribs

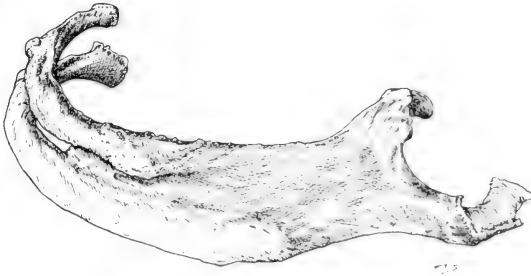


Fig. 21 Fused second and third ribs

and numerous Indian skeletons. In an examination of this large amount of material, Hrdlicka found only one case of junction of the third and fourth ribs but three cases of the much more common anomaly—fusion of the first and second ribs.

The specimen shown in figure 21 was taken from the cadaver of an old man, is composed of the second and third ribs. The rest of the skeleton was normal and the thorax was not unusually asymmetrical. Hence the anomaly was not very evident and my attention was called to it by Messrs. Supple and Tufts, two of our students. These two ribs which are of normal length were fused throughout three-fourths of their length. Nevertheless their relations were maintained exactly for they had independent cartilages and a short bifurcated medial extremity.

Unlike the specimens of fused first and second ribs described by Lane, the individual shafts are almost completely obliterated in the medial half of the fused portion, both within and without. This is true of a rhomboidal area lying within 8 cm. of the sternal extremity of the second and 7 cm. of the same extremity of the third rib. The intercostal groove is only very slightly preserved on the external surface of the lateral half of the fused portion. Since the fused portion extends to within 9 mm. of the medial extremities the appearance here is that of a bifurcated rib. The common shaft is 12.3 cm. long, the first rib 22.8 cm. and the second 26.5 cm. The width of the fused portion is 3.5 cm. laterally, and 4.3 cm. medially but the distance from the superior border of the first rib to the inferior border of the second rib at the medial extremities is 6.1 cm. This increase in width is due to the fact that the short non-fused medial extremities diverged rather markedly to meet the costal cartilages. The distance from the mid-point of the head of one rib to the midpoint of the head of the other is 2.1 cm. or normal.

In the lateral area of fusion the second rib is very definitely outlined for a distance of 4.3 cm. and the third for a distance 7.9 cm. Throughout this region of only partial obliteration of the shafts the intercostal region is marked by a broad deep sulcus and the outlines of the individual shafts are well preserved externally but not internally, where the intercostal region is marked by a decided ridge which gradually disappears toward the medial extremity. This rounded ridge looks not unlike the inner surface of a rib and gradually merges with the shaft of the first rib. The subcostal sulcus of the second rib is fairly well marked to a point of 2.5 cm. beyond where the fusion begins but that on the first rib extends 4.5 cm. beyond this point. The greater length of the sulcus on the first rib is due to the fact that it lies external to the bony union between the ribs for a distance of 4.3 cm. The intercostal nerves ran internal to the fused portion.

The superior margin of the flat, fused area is much thicker than the inferior, the two measuring 6 and 2.5 mm. respectively. The greater thickness of the superior margin is due to the better development of the bony area which would be comprised by the

second rib. In thickness, spacing and form where separate, and also in length where fused the ribs are entirely normal. The intercostal muscles are of course absent in most of the fused area which is covered by a strong intercostal membrane. The muscles are present, however, in the lateral portion of the fused area where the external portion of the bodies of the individual ribs are only partly unaffected by the fusion.²

BILATERAL ABSENCE OF THE EXTENSORES CARPIULNARES

Such a variation as the above is not mentioned by Le Double ('97) or in the larger handbooks such as those of Bardeleben and Poirier et Charpey. Neither do Quain nor any of the other German and English textbooks consulted speak of it. Gruber ('85) and Turner ('85), however, each reported a somewhat similar case. Gruber also says that he could not find anything like it in the literature.

Gruber observed the specimen reported by him in 1883, in the left arm of a young male 20 years old. It was the only specimen observed by Gruber in the course of the personal dissections of 600 arms! In this case the muscle was represented merely by a tendinous strand which extended, however, all the way from the external condyle to the fifth metacarsal. This strand which was fused with the fascia was 5 mm. wide above, 3 mm. wide and 1 mm. thick in the middle of the forearm and 4 mm. wide and 2 mm. thick at the point of insertion. The lower portion was supplied with a vaginal sheath and lay in an ulnar sulcus of normal dimensions. Other fairly common muscular variations were also present in this arm.

Turner who referred to Gruber said the case observed by him was wholly comparable to that of the former. In Turner's case also, the sulcus on the ulna was normal although the ten-

² Through the courtesy of Dr. Hrdlicka, it has since been my privilege to examine specimens of fused ribs in the Smithsonian Collection, and if I remember correctly, one of the specimens is very similar to what is here described. Through the courtesy of Dr. Lamb I was also enabled to examine a specimen of extended fused ribs in the museum of the surgeon-general, which belongs still lower down in the series of ribs.

dinous slip was only one-sixth the size of the normal tendon. Turner emphasized that such an anomaly was not mentioned by Macalister or Testut in their works on muscular anomalies, and that this case was the only one observed by him in thirty years' experience in the dissecting room.

A very careful examination of both upper extremities of this male subject by Messrs. Supple and Tufts who noted the absence early in their dissection, and by Professor Congdon and myself did not reveal a trace of these muscles. It seemed to me at first that a remnant of the tendon had fused with the internal lateral ligament of the wrist but a comparison showed that this ligament varies sufficiently in strength and distinctness to justify one in including those on these arms among the normal variations.

Although the *extensores carpi ulnares* muscles were completely absent in these arms the sulci on the dorsal surface of the distal extremities of the ulnae, in which they lie were nevertheless present and practically normal in size and depth. Hence these specimens again illustrate a certain independence in the formation of these and similar sulci, which nevertheless may be moulded by tendon pressure even if not primarily due to them. No other anomalies were found in this cadaver and no unusual strands or thickenings were present in the fasciae of these forearms. No modifications could be determined in the fifth metacarpals.

UNILATERAL ABSENCE OF THE TWELFTH RIB IN AN ORANG-OU-TANG

In a skeleton of an adult orang-ou-tang which seems to possess no other unusual characters the condition of the ribs deserves a word of comment. There are eleven pairs of ribs which look normal except for the development of a flat wedge-shaped square bony process one centimeter square on the anterior upper surface of the tenth rib. This process which is 3 mm. thick at the base arises from the superior costal border a few centimeters ventrally from the angle and must have come close to the inner surface of the superior rib although the latter bears no sign of such contact. Many of the ribs on both sides also bear triangular bony extensions along their inferior borders in the re-

gion of the angles a condition which may, however, for all I could learn, not be uncommon in the orang-ou-tang. The right transverse process of the nineteenth, or first lumbar, vertebra looked, practically like the corresponding process in human skeletons. The accessory and transverse processes were very well differentiated and were marked by a deep sulcus. The mammillary process though slender was well-developed and was separated by a broad deep sulcus from the inferior processes.

The left transverse process, on the other hand, had the typical form except that its extremity was pitted by a very definite articular cavity which received the head of the twelfth rib. The latter was 8.8 cm. long and the eleventh ribs 18.2 cm. It is particularly interesting that although the twelfth left rib did not articulate with the body of the vertebra but arose from the transverse process the right transverse process nevertheless showed no enlargement whatever and possessed a better differentiated accessory mammillary and transverse processes than most human vertebrae.

THE EFFECT ON THORACIC FORM OF COMPLETE DESTRUCTION OF ONE LUNG

Although the expression "One lung is gone" is not unfamiliar to medical students and to physicians who deal with large numbers of tubercular subjects it is necessary to recall that those words as customarily used by clinicians are not intended to be taken literally. Moreover, when one considers the great vessels in the root of the lung such a thing seems quite impossible. It would seem that death from hemorrhage must occur long before the great pulmonary vessels so near to the heart can be obliterated. Yet such a case recently came to my attention.

The cadaver was that of a man beyond middle age and nothing which could be identified with the naked eye as pulmonary tissue remained of the left lung. The remnant of the pleurae was roughened and thickened considerably and the remnant of the root of the lung, was represented merely by a short stub of dense connective tissue containing the sclerotic and wholly, or partially obliterated extremities of the bronchi, blood vessels and calcified lymph nodes. The left thoracic cavity contained

but very little coagulum and was otherwise completely empty. The left dome of the diaphragm was but slightly displaced. The fact that the mediastini and the contained viscera were displaced so slightly is likely explained by the thickening of the left parietal pleura long before the lung was completely destroyed.

I have not sufficient knowledge of either tuberculosis or pathology to venture a statement on the probable sequence of events in this case but the fact that the external form of the thorax was altered so slightly that it attracted no attention, does seem to indicate that not much tension could ever have been exerted on the thoracic wall as a result of an adhesive pleurisy. I recall that Hutchinson found that the long-expressed idea that tubercular individuals are flat-chested is erroneous but these findings do not imply that tuberculosis never affects thoracic form. Hutchinson found that the anterior posterior diameter in normal adult males between the ages of twenty to forty-four is 71 if the transverse diameter at the level of the nipple is taken as 100. This same diameter in 82 clinically tubercular subjects was found to be 79.5 and in 30 flat-chested individuals 80.

Instances in which the thoracic form has been profoundly changed especially in cadavers in which the ribs are markedly senile, are, of course, frequently seen in dissecting rooms where most of the material used is from the senile or tubercular or from the senile and the tubercular. But it is difficult to see how a lung can be completely destroyed by tuberculosis without an attendant pleurisy and consequent long-continued tension on the chest wall as a result of fibrosis. Nevertheless, the conditions were as here represented and there can be no doubt about the tubercular nature of the disease in this particular case.

OSSIFICATION IN THE ARACHNOID

The occurrence of small hardened areas in the spinal arachnoid is comparatively common in dissecting room cadavers. Because of this fact and also because these areas are usually very small I have in the past usually taken them for calcifications.

This assumption was based not only on their appearance but also on their physical properties. Some months since my attention was called to certain small areas of apparent fibrosis of the arachnoid and to other very much larger horny plaques. Mr. H. M. Winans and Miss Dorothy Wood, two of our medical students noticed these peculiar plaques in the lower dorsal region upon exposing the spinal cord. The largest of them has an area of 2.8 by 1.6 cm. and the next largest 2 by 1.4 cm. Both were less than 0.5 mm. thick, however, and were moulded so as to surround the dorsal portion of the cord in the region over which they lay. They were adherent neither to the pia nor dura. Both these large plaques and the smaller similar ones were very flexible and quite translucent.

In cutting off a portion for microscopic examination it was evident that these plaques both had the hardness of bone rather than of horn and that they lacked the friableness or at least the brittleness of calcified areas. Examination of paraffine sections showed that these areas were composed of lamellae laid parallel to the flat surfaces of the plaques. The lamella contained some rather atypical bone corpuscles and were penetrated at intervals by perforating or atypical Haversian canals which here and there opened upon the surface. Several small narrow cavities were also found and the internal surface also showed a narrow calcified layer. The outer portion, on the contrary, was formed by a single layer of investing cells. The body was that of an Irish woman of 75 who had died of arterio-sclerosis and in whom the porencephalic brain referred to on page 76 was found.

In view of the close relationship between fibrous tissue and osteogenetic processes, the mere presence of bone in the arachnoid is nothing particularly surprising. A discussion of this subject in man and animals with references to the literature and numerous interesting personal observations is given by Cushing and Weed ('15).

CUTANEOUS PIGMENT IN THE SPERMATIC FASCIA

Although small quantities of cutaneous pigment are found in the cutis of man I am not aware that the so-called superficial fascia has been found pigmented. It is true that Toldt ('13) found that the epidermis in the dark skin spots in *Macacus inuus* and *Cebus libidinosus* contained very little pigment and the deep layers of the corium much, but this pigment was intra and not intercellular for it was contained in large branched pigment cells. Toldt also emphasizes the relatively great pigmentation

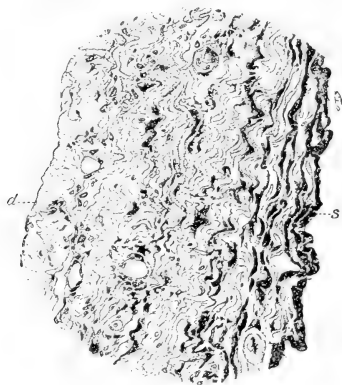


Fig. 22 Spermatic fascia from *Didelphys virginiana*

in the corium of these mammals as compared to the lower vertebrates. Adachi ('03) on the other hand concluded that there is a correlation between the intensity of epidermal and dermal pigmentation but found that the quantities of pigment contained in the cutis were relatively small. Breul ('96) and Frederic ('06) strangely enough found pigmented places in the corium most common in the relatively unpigmented regions.

In figure 22 a portion of the spermatic fascia of an opossum is shown. The surface marked *S* is the superficial one and that marked *D* the deep one. As shown in this figure large masses of pigment are scattered about in almost the whole of this fascia

the superficial layers of which contain thick layers of pigment. In addition to these larger masses pigment granules were also scattered about in the fascia but practically all of the pigment was extra-cellular. Although I am not here concerned with the finer questions relating to the origin and occurrence of normal skin pigment there can be little doubt that we are dealing with normal cutaneous pigment. The gross character of the overlying skin alone makes this certain.

The scrotum and the para-scrotal skin of this opossum was intensely bluish black. The color of this area made it look like 'tache bleuatre,' the peculiar shade being of course due to corium pigment. Since similar pigmented areas as found in the scrotal region of this opossum are relatively common in mammals and since other similar areas were found on this very animal the scrotal pigmentation attracted no special attention until the skin and some of the superficial fascia had been reflected, butcher-wise, and the testes were seen to be just as intensely bluish black. On incising the spermatic fascia and reflecting the parietal tunica vaginalis with it, the testes were seen to have the usual color.

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THE HYPOPHYSIS OF THE GUINEA PIG

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SEVEN FIGURES

The guinea pig was used as the object of this study because it is readily obtainable and also because though thousands are used yearly for experimental purposes, this animal has been neglected from an anatomical standpoint. The only reference found to the anatomy of the hypophysis of the guinea pig, was one by Oppel ('14) who referred in a few lines to its general relations and another by Paulesco ('08) who quotes Fischera in regard to the weight of the gland.

The general relations of the hypophysis of the guinea pig are very similar to those existing in other vertebrates and its microscopic structure is quite similar. The most striking difference found was the presence of cilia in the cleft and also in the epithelial cysts or vesicles of the glandular portion. According to Trautman ('09), ciliated epithelium has been reported in the vesicles in the hypophysis of man and the rabbit, but Trautman was unable to find any in the cysts of any of the domestic animals studied by him. These included the horse, ass, cow, calf, sheep, goat, hog, dog and cat, and although he could not be certain Trautman thought he saw ciliated epithelium in the lining of some portions of the cleft of the pituitary of the hog. In the guinea pig this ciliated epithelial lining of both the cleft and the vesicles was found uniformly present somewhere in all the specimens examined, but it was not present in all cysts nor did it completely line the clefts. Within the latter, in fact, it did not appear at all on the side adjacent to the pars intermedia and only on portions of the opposite side. These portions are easily recognizable even under low magni-

fication by the large, clear, columnar cells, which often project into the lumen, as little mounds. In some cases, however, the ciliated portion of the lining epithelium extends over considerable stretches. The fact that ciliated epithelium is present in both the cleft and in the closed vesicles of the epithelial portion seems to indicate that their origin is the same and that both undoubtedly are remnants of the lumen of the epithelial pouch (Rathke's pouch) developed from the buccal epithelium. In several cases, for example, it was possible to trace the lumen of typical vesicals to the lumen of the cleft.

METHODS

In every case a portion of the brain was removed with the hypophysis in order to retain its relations, particularly with the third ventricle. All microscopic preparations were cut serially in paraffine. Sections cut sagittally in the cranio-caudal plane were used mostly since they best show the relations of the different parts. Zenker's fixative, followed by methylene blue and aqueous eosin were found to be the best general fixative and stain. Flemming's and Orth's fluids were also used. The phosphotungstic acid-hematoxylin stain was found valuable in staining neuroglia and ependymal fibers and cells, and also reticular connective tissue. Next to the methylene blue and eosin, aqueous eosin and iron-hematoxylin were found to be most satisfactory. The latter stains the cell boundaries especially well (figs. 3 and 4).

OBSERVATIONS

The hypophysis of the guinea pig is comparatively large. Measurements made upon three freshly killed adult animals show that it weighs between 22 and 32 mgm., varying somewhat in different specimens. Paulesco ('08) quotes Fischera as giving the weight of the gland as 0.015 gram, but he does not state whether his data were derived from specimens of fresh or preserved glands. By weighing the brain also in each case,

it was calculated that the gland represents between 0.52 per cent and 0.70 per cent of the total brain weight.

The measurements made on formaline fixed glands as to size gave the following averages:

	<i>mm.</i>
Transversely (widest part).....	5
Cranio-caudally (widest part).....	4.2
Dorso-ventrally (widest part).....	1 $\frac{2}{3}$

The brain proper, including the cerebellum had the following average dimensions:

	<i>cm.</i>
Transverse (widest part).....	2.1
Cranio-caudally (widest part).....	3.1
Dorso-ventrally (widest part).....	1.1

From the above measurements it will be seen that the hypophysis is comparatively large; that its transverse diameter is about one and a fourth the cranio-caudal diameter and that it is much flattened dorso-ventrally.

The general relations of the gland to the brain are indicated in figure 1, which is a semi-diagrammatic sketch of the inferior surface of the brain with the dura mater removed. The hypophysis lies just posterior to the optic chiasma in an almost horizontal plane, being practically parallel to the plane of the base of the brain. The cerebrum, descending on either side, forms a considerable fossa in which lies the pituitary with its dural sheath, the blood vessels and sinuses. Laterally on either side and partly covering it, lie the two very large fifth nerves with their ganglia. The gland is attached to the tuber cinereum by a comparatively long stalk which expands posteriorly forming the pars nervosa. In the figure only a small portion of the pars nervosa is visible posteriorly, from the inferior surface, for the greater part of it is covered by the pars glandularis. The dorsal end of the gland is free except where it is attached to the brain by the blood vessels which enter its superior surface.

The dura mater forms a distinct pouch or pocket for the whole gland and is much thickened behind where it is often cartilaginous in old animals. In section it shows that it is split off from the main dural covering of the brain, but the cavity of the

pocket is nevertheless continuous with the main subdural space. The gland is loosely attached to the dura by little strands of arachnoid tissue but the space between is easily penetrated by fluids.

A large vascular sinus surrounds the posterior end of the gland in a horizontal direction between the two layers of the dura mater which is here split to form the dural pocket. Its walls are thin and composed only of a thin layer of fibrous

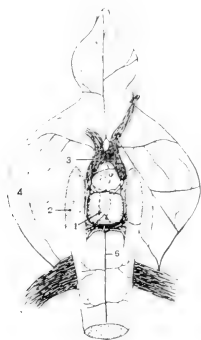


Fig. 1 Diagrammatic sketch of the inferior surface of the brain of the guinea pig showing position of the pituitary gland and its relation to the surrounding structures. 1, pituitary; 2, divided fifth nerve and semilunar ganglion; 3, optic chiasma; 4, cerebrum; 5, small portion of pars nervosa projecting from above the pars anterior; 6, basilar artery.

tissue lined by endothelium. The lumen fits the space between the two dural layers and therefore has a general triangular shape but it is often irregular. Sometimes it partially replaces the subdural space extending anteriorly over the superior or inferior surfaces or over both for varying distances. In these cases the fibrous layer is inseparably fused with the capsular layer of the gland. In animals in which the arterial system was injected with India ink this sinus shows very distinctly as a black line extending around the posterior end of the gland.

About the middle of the posterior end of the gland there is a smaller connecting sinus which extends directly posteriorly within the dura mater, presumably forming a connection with some other blood vessel. There is nothing to indicate the function of these venous vessels but it seems probable that they drain some portion of the pituitary, probably the meninges. The circular sinus of the pituitary was found in every series of sections observed but the sinus passing posteriorly could only be seen when the knife happened to pass parallel to its lumen. That they were sinuses and not lymph channels, was shown by the fact that they were often engorged with blood.

The hypophysis with its dural covering, lies upon the posterior third of the body of the sphenoid bone. The sella-turcica is represented only by a small fossa, at the bottom of which there is usually a small opening. If closure of the cranio-pharyngeal canal is partly or wholly prevented by the persistence of the epithelial stalk, this minute aperture, opening into the bone may thus remain. In no case, however, had the stalk retained its epithelial structure and in most cases no trace of it could be found. Within the sphenoid the canal is more or less obliterated by encroachment of the bone. On the pharyngeal or inferior surface a second foramen which transmitted the buccal end of the stalk is sometimes seen.

The relations of the different parts of the hypophysis can best be understood by referring to figure 2. The epithelial portion lies upon the inferior surface and is represented by the darkened area. In section it appears smaller than the light area or pars nervosa but, owing to the fact that it extends so far laterally, almost surrounding the nervous portion here and anteriorly actually doing so (9) it is really considerably—probably a third—larger than the pars nervosa. It extends as a thin epithelial layer anteriorly over the neck and also over the base of the tuber cinereum. For this reason this part is called the pars tuberalis (4) by Tilney '14. Tilney claims for it a separate development from Rathke's pouch.

The infundibulum cerebri (fig. 2) is a continuation of the brain backwards and slightly downwards. It originally con-

sisted of brain tissue but apparently degenerates laterally into a connective tissue mass containing a few epithelial cells. Colloid is found in the meshes but this probably has its source in the pars intermedia. The infundibulum which is funnel-shaped near the base of the brain becomes comparatively small at its neck and at the distal end widens out into a club-shaped mass, which forms the main portion of the pars nervosa. In some animals, notably the cat, the posterior lobe is hollow, but in the guinea pig it is nearly solid, the opening from the third ventricle ending in the neck as seen at 5 and 6 in the figure. The

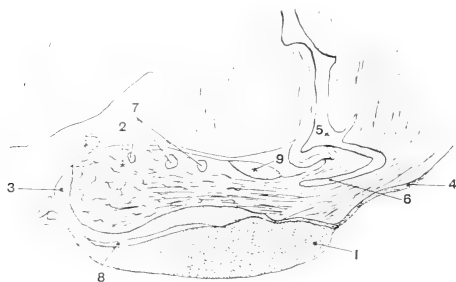


Fig. 2 Median sagittal section through pituitary of adult guinea pig. (Semi-diagrammatic.) 1, pars anterior; 2, pars nervosa; 3, pars intermedia; 4, tongue-like process of pars tuberalis of intermediate lobe; 5, third ventricle; 6, recessus infundibuli; 8, cleft; 9, portion of intermediate lobe which completely surrounds the pars nervosa.

end of the cavity usually enlarges to form a small irregular bulb. In most cases the nervous lobe is not absolutely solid but contains cysts (7) of varying size which are presumably remnants of the foetal condition in which the lumen of the third ventricle extended down into the body of this lobe.

The pars glandularis is differentiated into two portions, the pars anterior (1) and the pars intermedia (3). These parts are partially separated by a cleft (8) which is described as the remains of the lumen of Rathke's pouch by Tilney '14, Herring '08 and also by others.

THE PARS ANTERIOR

The pars anterior is composed of columns of epithelial cells with blood sinuses separating them (fig. 3). Presumably, the secretion from the cells is absorbed directly into the circulating blood. The cells of the pars glandularis are large and glandular and have different staining characteristics. Usually there is a distinct investment of basophilic cells upon the peripheral surface, the interior being composed mostly of acidophile

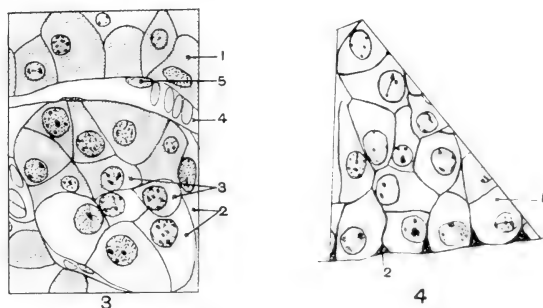


Fig. 3 Drawing made from section of pars anterior of guinea pig's pituitary. Stained deeply with iron hematoxylin and aqueous eosin. 1, deeply staining acidophile cells with large granules; 2, nearly clear cells slightly stained (neutrophiles); 3, intermediate stages; 4, blood sinus containing blood corpuscles; 5, large nucleus of cell lining blood sinus.

Fig. 4 Section of pars intermedia of pituitary of adult guinea pig stained with iron hematoxylin and aqueous eosin. 1, typical cell with fine granules; 2, branched supporting cells. The base of the drawing is the part adjacent to the cleft.

cells. The nuclei are large and distinct and show many variations in regard to the chromatin. A peculiarity of this portion of the gland is the marked richness of the blood supply. In this respect it differs remarkably from the pars intermedia in which the blood supply is very scanty indeed.

There has been much discussion of the significance of the different staining characteristics of the cells of the anterior lobe but it is not necessary to give a historical account of the

different opinions here. Herring '08 considers the subject in some detail. It suffices to say that some authors describe three and four different kinds of cells according to differences in staining and ascribe different functions to each. Others claim that the different staining characteristics are due to differences in secretory activity of the cells. My observations upon the guinea pig tend to confirm the latter view. The different staining characteristics are, of course, always present to a very striking degree. With methylene blue and eosin, some cells are notably acidophilic, others notably basophilic. But between these two extremes all gradations in staining were found (fig. 3). Nor could any uniform difference in the shape and size of the differently staining cells be seen. The structure of the nuclei is apparently the same and they are subject to the same irregularities in size in the different types of cells.

In addition to the cells which constitute the anterior lobe proper which were described above, there are reticular cells which can be recognized by their opaque and uniform stain and also the cells lining the bloodvessels.

The pars anterior is nowhere in contact with the pars nervosa for a portion of the pars intermedia always intervenes. No colloid is found between the cells of the pars anterior but the vesicles especially characteristic of the pars intermedia were also occasionally found.

The portion of the anterior lobe which lines the cleft is composed of a single layer of epithelial cells. These cells are sometimes irregular, cuboidal or columnar. The columnar cells are usually ciliated. They are most regularly found at the posterior end of the cleft but may appear in little mounds anywhere along the cleft on the side adjacent to the anterior lobe (fig. 6). Beneath this layer of epithelium there are usually found true capillaries and spaces which may or may not be lymph spaces as suggested by Herring. In this portion connective tissue cells are most abundant.

THE PARS INTERMEDIA

The pars intermedia or juxta-neural portion is described as arising from the same source as the pars anterior, namely from Rathke's pouch. In a guinea pig at birth there is little differentiation between these portions but in the adult the difference is striking. There are no intensely acidophilic cells in the pars intermedia (fig. 4). At the point of junction at either end of the cleft, the change from the intensely red cells in cases of eosin staining, usually found in that region to the smaller lightly staining cells of the pars intermedia is quite abrupt. Herring ('08) found that this is not always the case in the cat, but in the guinea pig the abruptness of the transition was quite striking in every series of sections examined.

The pars intermedia partly invests the pars nervosa. The whole of the inferior and lateral surfaces and the greater part of the superior surface of the latter is covered by a comparatively thick layer of epithelial cells. However, posteriorly and superiorly there always is a region in which there is no epithelial investment. Here many of the bloodvessels of the pars nervosa enter, and just within the nervous substance, lie the remnants of the lumen developmentally continuous with the third ventricle, which are shown in figure 2. It may be significant that these remnants, or residua of the lumen, are always found in that region. They often get so near the surface that only the general connective tissue covering of the gland lies between them and the subdural space. In no case, however, could an opening from one into the other be found.

The cells of the intermediate are slightly smaller than those of the anterior lobe. Their nuclei are perhaps a little smaller but they have the same general appearance and stain uniformly. They are slightly acidophile but with an excess of basic stain will stain basic. Sometimes they are finely granular but they never contain the coarse granules of the acidophilic cells of the anterior lobe. Connective tissue cells are quite abundant especially along the cleft, where the branched supporting cells are much in evidence (fig. 4). According to Retzius who

is quoted by Herring, these cells in the cat "are for the most part small and thread-like and reach through the whole border. Others do not pass through but are branched. The cell nuclei are often placed near the outer end, while the inner end widens to a three-cornered foot, which is placed against the nervous tissue of the posterior lobe." With the stains used, it was impossible to confirm these statements satisfactorily in the guinea pig, but supporting cells extending from the cleft towards the pars nervosa were seen. They were branched and unbranched, and their enlarged nuclei usually were situated between the extreme ends of the cells lining the cleft or just within their outer border. In the former case they appear as little black triangles (fig. 4) which were moulded to fit the inter-space between the cells while, in the latter, they were much elongated and usually more transparent.

A very striking difference between the pars anterior and pars intermedia is the great wealth of blood supply in the one and its almost total absence in the other. A few capillaries only were found in the posterior portion of the pars intermedia but in the anterior portion, especially where it caps the pars anterior in the region of the neck, a great many were found which pass into the large arteries and veins of the neck of the infundibulum. In this case, however, the capillaries are comparatively large and apparently furnish only a small supply of blood to the surrounding tissues. However, at the junction of the pars intermedia and the pars nervosa there is usually an abundant vascular supply. In sections a vessel containing erythrocytes can nearly always be identified between these two portions of the hypophysis.

THE CLEFT

The cleft which partially separates the pars anterior from the pars intermedia, is a persistent portion of the lumen of Rathke's pouch. Upon the further separation of the infundibulum from the pharynx by the growth of the intervening parts, the infolding pouch is described as being drawn out into a long tube connecting the bulbous expansion of the cranial end with

the pharynx. The tube ultimately disappears, but the bulbous expansion partially remains and is represented in the adult by the cleft of the pituitary. It is always present in the guinea pig but it is not nearly so extensive comparatively as in the cat. Posteriorly and laterally it is often branched and irregular. In these localities cysts are most often found, especially at the posterior and anterior ends of the cleft. These evidently are portions cut off from the main cleft, which retain the original lumen as the lining of the cysts. The cleft is lined superiorly and posteriorly by the epithelium of the intermediate lobe; inferiorly and anteriorly by the cells of the anterior lobe. These lining cells are sometimes high columnar and ciliated and are usually only one cell deep.

The 'colloid' vesicles or epithelial cysts or acini,—various names are used synonymously by different authors—are especially characteristic of the pars intermedia. These cysts are of varying size, being usually largest and most numerous at either end of the cleft and, as pointed out above, are without doubt, merely portions of the original epithelial pouch, which have been cut off from the original lumen in the process of its partial obliteration. The cysts are surrounded by a single layer of epithelium. In some cases this is cuboidal but in others it is high columnar and ciliated. Some cysts contain both kinds of cells. One end of a cyst may have low cuboidal cells which further on, may gradually assume the typical high columnar form. The fact that portions of the epithelial lining of these cysts are usually ciliated and that the same type of ciliated cell is also found lining the cleft, alone strongly suggests a common origin. But what is more convincing is that in a few cases it was found possible to trace the lumen of the cyst to the cleft. Figure 5 is a drawing, outlined by the aid of a camera lucida of such a typical epithelial vesicle, the cavity of which could be traced to a direct connection with the cleft. The cell boundaries were not well-defined owing to the fact that the section was stained with methylene blue and eosin, which, though giving a high transparency and clearness does not stain the cell boundaries well. The cilia were quite clear and dis-

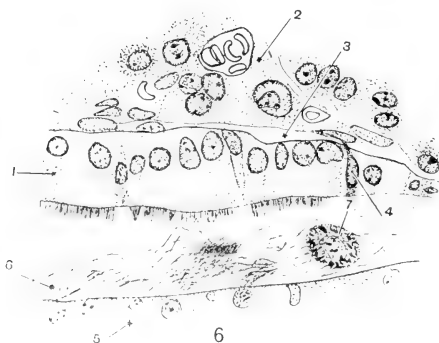
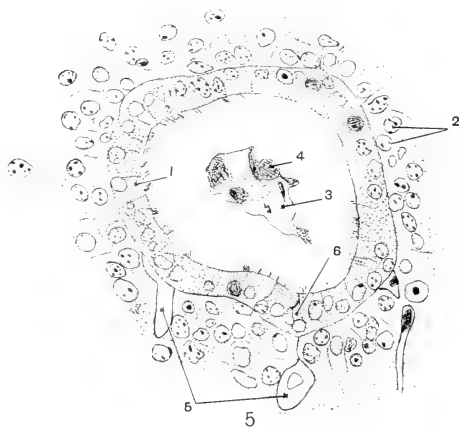


Fig. 5 Drawing of a cyst from the pars intermedia. 1, ciliated columnar cells surrounded by nuclei (2) representing cells of pars intermedia. The cell boundaries did not stain; 3, granular contents containing (4) a degenerating cell; 5, blood vessels of pars intermedia; 6, blood vessels entering between cells of vesicle.

Fig. 6 Portion of cleft lined by ciliated epithelium. Stained with methylene blue and eosin. 1, ciliated columnar epithelium; 2, cells of pars anterior; 3, blood vessel usually present above the row of lining epithelium; 4, supporting cells. Note absence of cilia at this point; 5, location of pars intermedia; 6, granular fibrinous mass suggesting coagulum containing (7) a very large degenerating epithelial cell, the nucleus of which still contains characteristic granules.

tinct and normal looking in this specimen. In some cysts they are formed into little tufts, one for each cell, while in others they seemed to project quite uniformly from the whole circumference of the lining epithelium. In other cases they were pulled away by the contraction of the contents of the cysts during fixation.

These vesicles were rarely found in the pars nervosa also, but in no case was there ciliated epithelium lining them. In two specimens typical ciliated cysts were found lying practically wholly outside the gland in the region of the original attachment of the epithelial pouch at the extreme anterior end of the glandular portion in both cases. They were probably persistent portions of the stalk. In one of the two cases the cyst could be traced as far as the dura where it merged with the connective tissue, the bounding cells showing marked signs of disintegration.

Many authors have described colloid as existing in these cysts and also in the cleft, but none could be found in either place in the guinea pig. Its absence did not seem to be due to defective fixation or staining, for the colloid found elsewhere in the pars intermedia and particularly in the neck of the infundibulum was both well-fixed and well-stained. Most of the vesicles contained a granular mass without any particular structure evidently derived from degenerated epithelial cells, for cell detritus was often found in the mass and cells apparently being detached from the walls of the cyst were also seen. This cell detachment did not seem to be an artefact for it was found many times in areas where the other cells showed no signs of damage and were fixed perfectly. The degenerating cells, in some cases, were drawn out into a pear shape, the small end of the pear alone being still attached.

The degenerating cells in the cleft seemed to come from the pars glandularis. Some of these cells still retained their acidophile properties but did not show clear-cut granules. They contained a substance resembling mucous in appearance and staining qualities, and wholly unlike the colloid found in the posterior lobe.

THE PARS NERVOSA

There has been very much discussion of the structure and function of the pars nervosa. Some writers, notably Berkley ('94) have described nerve cells. Berkley described several different kinds of cells but the work of later investigators seems to disprove the presence of any nerve cells whatever and it seems that the earlier authors probably mistook the ependymal and connective tissue cells. The posterior lobe seems to be made up of a mass of interlacing ependymal and neuroglia cells and fibers running in a general longitudinal direction. Occa-

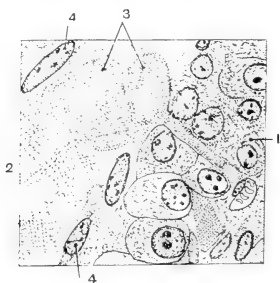


Fig. 7 Section through part of pars intermedia and pars nervosa at their point of junction. 1, cells of pars intermedia; 2, pars nervosa; 3, colloid appearing both in pars nervosa and pars intermedia. Note the granular appearance of the colloid; 4, neuroglia nuclei.

sionally there are little clumps of what may be epithelial cells, though, in the guinea pig, they are small and not characteristic. For information on the pars nervosa reference is made to Herring. Herring worked upon the cat which is an animal more satisfactory for this study than the guinea pig because the pars nervosa of the cat is hollow and its lumen directly continuous with the third ventricle.

In the guinea pig there are few inclusions of epithelial cells in the pars nervosa. Figure 7 shows a portion where the epithelial cells had pushed their way slightly into the substance of the nervous portion. It was the only place in the section.

Here, as indicated, the colloid material of the pars nervosa and that of the pars intermedia are directly continuous. In fact, one mass of colloid lies partly in both portions. It has the appearance of having been fixed while passing from the one into the other. There were few cells in the pars nervosa that could possibly be secretory. Hence it seems reasonable to suppose that the colloid secretion was passing from the pars intermedia into the pars nervosa. Physiological experiments also support this view. Moreover where the tongue-like process of the pars intermedia extends over the base of the tuber cinereum which is not connected with the pars nervosa of the pituitary, the colloid is often found in the substance of the latter structure. In this region there are no closed vesicles as in the pars nervosa and little evidence of any epithelial inclusions. This would also seem to exclude the theory that in the guinea pig the colloid results from the degeneration of epithelial cells in the pars nervosa, and also the possibility of the secretion being produced in the pars nervosa. Colloid is often found in greatest abundance in the neck of the infundibulum. The structure of the nervous portion does not seem to account for its importance and since the epithelial cells of the middle lobe have been in contact with it from an early stage of development it would seem that its importance lies in this relationship. That is, that the secretion from the intermediate lobe passes through the pars nervosa into the brain substance perhaps on its way to absorption by the cerebrospinal fluid.

In the guinea pig the ependymal cells of the different parts of the nervous portion seem to vary. In the neck and therefore near the end of the recessus infundibuli they are quite distinct and can be traced for some distance gradually merging into ependymal fibers. In the body of the lobe, they seem to lose much in staining properties and therefore in distinctness. There are, indeed, many fibers running in bundles in all directions but they are smaller and are indistinct while the cells themselves can hardly be recognized although their large nuclei are always very striking.

The cysts found in the pars nervosa of the guinea pig presumably are the remains of the original cavity of the recesses infundibuli. They are lined with a thin layer of connective tissue, which gives no clue to its origin. In different animals, there is a variation in their size. In one specimen the opening was quite large and could be traced very nearly to the end to the retained recess in the neck. These cysts are usually filled with a granular mass somewhat similar to that found in the colloidal cysts of the pars intermedia. A lining membrane is not always present.

In every series of those sections in which the dural pocket was retained with the gland the so-called parahypophyses were found. Their most usual situation was in that portion of the dura which covers the inferior surface of the neck or infundibulum of the gland, but they were found anywhere in the dural covering of that structure. They are glandular in structure but are much degenerated and probably nonfunctioning.

In one series of sections a vesicle of the pars intermedia situated near the neck of the gland, was traced across the subdural space into the dura and in later sections appeared as a characteristic parahypophysis. Since these vesicles are probably unobliterated portions of the lumen of Rathke's pouch, it follows that these pseudoglands are merely remnants of the walls of the pouch for the two are continuous.

In two of the above series of sections, a peculiar cartilaginous structure was found, situated between the dura mater and the sphenoid bone. It had practically the same appearance in both cases in which it was found. It consisted of a comparatively long partly tubular cartilaginous structure, extending from the anterior portion of the gland to beyond the posterior end by about one third the length of the gland. That side of the cartilaginous tube adjacent to the sphenoid bone was partly missing and the remaining portion showed defects in staining and possessed no perichondrium. There was no evidence of any tearing for the edges were smoothly rounded off where the defect in the tube commenced. The cartilage next the dura was about as thick as that structure and was apparently normal. The

perichondrium was in places fused with the dura. The lumen of the cartilaginous 'tube' was lined by columnar ciliated epithelium. In some places this lining was much degenerated, but at others and particularly at either end, the epithelium was quite characteristic of the epithelial lining of the mucous membrane. Moreover, there were numerous mucous glands whose lumen could be traced directly into that of the tube, leaving no doubt as to the origin of epithelial lining from mucous membrane. I saw no evidence regarding the probable origin of the cartilage, however. In one of the two series referred to above, the anterior end of the tube could be traced through the sphenoid bone to the pharynx where it apparently stopped.

SUMMARY

1. Ciliated epithelium was found to line some portions of the side of the cleft adjacent to the pars anterior but was never found on the opposite side.

2. The so-called 'colloid vesicles' of the cysts of the pars intermedia and occasionally of the pars glandularis are usually lined with ciliated epithelium.

3. From the above two considerations and also from the fact that in the adult guinea pig, the lumen of an apparent cyst can sometimes be found continuous with the lumen of the cleft, the conclusion that these cysts and the cleft have the same origin is justified.

4. No colloid-like substance was found either in the cleft or in the vesicles. Both contained granular substances embedded in what stained and appeared like mucous, and which occasionally contained degenerated epithelial cells. These cells seemed to have broken off from definite regions of the lining.

5. The only substance resembling the colloid of the thyroid gland was found in the pars intermedia and nervosa, especially in the neck of the latter. Also, very often it was found in that portion of the tubercinereum covered by epithelium. This substance occasionally has a granular and hyaline appearance, thus differing from the colloid of the thyroid.

6. Apparently the colloid just described is a secretion of the pars intermedia passing via the pars nervosa into the brain substance, perhaps to be absorbed into the cerebro-spinal fluid.

In concluding I wish to express my thanks to Professor Meyer for his assistance, helpful suggestions and criticisms in the preparation of this paper.

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THE THORACIC DUCT OF THE ADULT GUINEA PIG

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THIRTY-FOUR FIGURES

There are comparatively few reports in the literature covering considerable numbers of thoracic ducts in different animals. Davis ('15) described and pictured the thoracic duct of man in 22 cases, he being the first to describe and picture over 20 thoracic ducts completely in any one animal. McClure and Silvester ('09) described the termination of the thoracic duct in 50 animals of 25 different species and pictured 31 cases. Parsons and Sargent ('09) described the thoracic duct in man, from the middle of the thorax to its venous connection, in 40 cases, Verneuli ('53) described its termination in 24 cases and Wendel in 17 cases in man. Other investigators have reported abnormal cases but the number reported by each individual has been comparatively small, and will be mentioned later.

In this article the author has given the results as he has found them without endeavoring to explain any of the findings from a developmental point of view. The development of the lymphatics of the guinea pig is unknown and in those animals in which it is known it is still a matter of controversy. A personal investigation of its development in the guinea pig was for several reasons impossible.

In the course of the work 34 guinea pigs of adult size (of these 20 or 58.8 per cent were males and 14 or 41.1 per cent were females) were used. Since they were obtained from different sources, they were not in-bred and the chances of any inherited similarity of variation were thus eliminated.

METHOD

Each animal was killed by the use of ordinary illuminating gas. The guinea pig is extremely susceptible to the gas. Each animal was taken from the jar at once and injected immediately with India ink. For injection a 2 cc. hypodermic syringe with a fine needle was used and from 1 to 2 cc. of diluted india ink was injected subcutaneously into the pad of each hind foot. Then pressure was applied to the pad by the hand and the legs worked and massaged. An incision was then made through the skin the whole length of the ventral surface of the trunk in the median line, and from this an incision was made out to each of the four limbs. The skin was then reflected. The abdomino-inguinal lymph nodes were then visible, being partly or completely colored by the india ink. These lymph nodes appeared in groups of three or four on each side. Into these nodes I injected about 0.5 cc. of India ink. The abdomen was then opened by a median incision and a transverse incision. By displacing the intestines two large nodes were visible, one on each side in relation to the iliac blood vessels. These nodes too were already colored with the ink which had passed through them and up into the thoracic duct which showed very distinctly. Into these two large external iliac nodes about 0.5 cc. of ink was injected. I also injected about 1 to 2 cc. of ink into the mesenteric nodes. I first injected the smallest which lie near where the colon joins the small intestine. The ink flowed along a number of channels into several large nodes near the root of the mesentery. By all of these injections a large amount of ink was put into the thoracic duct. I immediately tied off the two internal and two external jugular veins, the subclavian veins, and the superior vena cava, and thus kept the ink from passing into the venous system. The animals were then put into 10 per cent formalin with as little handling as possible and dissected after twenty-four hours or more. Before preservation a preliminary examination was always made for fear of some of the injection fading out. The dissections were performed with the aid of a lens when necessary.

Lewis ('05) in working out the development of the lymphatic system in the rabbit, began with a 21 mm. pig, the species used by Sabin, to test his technique. In order to test or control my technique, I injected a young cat in a similar manner and after it was fixed, dissected it. I found the duct beginning at a large cysterna chyli continuing as a large single duct for some distance. It then divided into two vessels for a distance of several centimeters and again united and joined the left external jugular vein. Davison ('10) describes and pictures a corresponding duct in the cat.

GENERAL RESULTS

The injected ink from the hind foot came to the abdomino-inguinal nodes in several channels. In a number of the cases, the ink would pass into the first node and then on to the next. In many cases some of the ink passed the next abdomino-inguinal node by a vessel running around it and thus passed along faster than that which went through the node. But this shunting never took place in the two large iliac nodes which were large and oval with from one to three afferent lymph vessels entering the distal end and with one and sometimes two efferents. In every case the ink passed with perfect ease through these nodes and up into the lumbar plexus and then into the thoracic duct. It never backed up into the mesenteric nodes.

In nearly every case while injecting the mesenteric nodes about the same time that a node became completely black, the ink would quickly back up all the veins that extend between the node to the intestine. This retrograde injection of the veins happened in every instance in the injection of the large nodes near the root of the mesentery and confirms the observation of Meyer ('14). Baum ('11) has endeavored to explain similar occurrences in other animals. This retrograde injection of the veins never happened with any of the other nodes injected, as the inguinal or external iliac nodes.

In every case the lymphatics from the kidneys which connected with the lymphatic plexus around the abdominal blood vessels where the renal blood vessels come off, were injected

back into the kidney. Between the two kidneys and dorsal to the blood vessels two to three nodes were always present on each side and sometimes also one or two in the mid-line. The nodes on the right side nearly always appeared to be the larger.

THE THORACIC DUCT

The thoracic duct in the guinea pig is not a single but most usually double, consisting of a right and left duct lying at the respective sides of the thoracic aorta. At the beginning of the thoracic duct in the thorax there was in more than half the cases a dilation which will be spoken of later. The thoracic duct arises in the abdomen from a plexus of lymph vessels lying dorsal to the abdominal aorta and the inferior vena cava. A plexus of lymph vessels lying ventral to these vessels connects with the dorsal plexus. The latter may contain several lymph nodes (fig. 12) and is connected with the several intestinal trunks, which join with this plexus and with the nodes on either side of the aorta. Over the ventral surface of the lumbar portion of the inferior vena cava and the aorta there is found a plexus of lymph vessels connecting caudally with the iliac nodes and cranially with the superior lumbar abdominal plexus. This plexus is connected on each side of the blood vessels with the inferior dorsal lumbar plexus. In the figures the ventral plexus of the lumbar region which was rather indistinct has not been shown because it was dissected away but the cut connections with the dorsal lumbar plexus can be seen at x in the figures.

In the thorax a very definite plexus exists between the two ducts. The thoracic plexus which, however, lies wholly dorsal to the aorta usually extends between the 8th and 12th or 13th thoracic vertebra. In a number of cases this thoracic plexus was not continuous with the superior dorsal lumbar plexus, but was joined by one or more channels. In no case was there any vessel that was injected which ran ventral to any part of the thoracic aorta.

The right and left ducts continued on their respective sides of the aorta cranially until at about the 5th to 6th thoracic

vertebra where the right duct passed cranially behind the aorta over to the left side and usually joined the left duct between the 2d to the 1st thoracic vertebra. Before the main union of the right and left ducts, the left passes over the left innominate artery, but under this artery there was usually a cross connection between the two ducts. The united ducts continued behind the left innominate vein and entered it usually at the junction of the internal jugular or between that of the internal and external jugular veins, that is at the jugular angle.

Davis ('15) described nine types of ducts and Lane ('47) three. These classifications depend upon whether the duct is double or single, whether it terminates in the venous system on the left or the right side or on both sides, and in the case of a single duct, upon which side of the aorta it lies, thus making nine types in all. In man, Davis found 6 out of 22 cases, or 27 per cent in type 2, that is, possessing double ducts which unite before terminating on the left side. In the guinea pig I found 30 out of 34 cases (figs. 1 to 34, except 7, 12, 20 and 23) or 88.2 per cent of this type. Lower ('80) and Nuhn ('49) have also described similar thoracic ducts in man. Fleischman has regarded as normal in man, ducts which subdivide into two trunks which are frequently connected. Type VI in which the thoracic duct is single and lies to the right for the most part and later turns to the left between the 3rd and 5th thoracic vertebra and opens into the venous system on the left, is described as normal for man by anatomists. Davis found this type to occur in 63.6 per cent of his cases. In the guinea pig I found 1 case (fig. 12) out of 34 or 2.9 per cent belonging to this type. In type IX in which the thoracic duct runs on the left side for its entire extent and empties into the venous system on the left, Davis described 1 case or 4.5 per cent of his cases. Cameron ('03) also described a similar case in man. In the guinea pig this type occurred in 3 cases (figs. 7, 20 and 23) out of 34 or in 8.8 per cent. In all 3 cases of this type it is interesting to note that the right thoracic duct extended cephalad to about the 9th vertebra, and that the usual plexus between it and the left thoracic duct was present. It ended blindly in only one case (fig. 7), and in the other two

it turned over towards the left. In one of these (fig. 20) it even ran some distance caudally after turning towards the left.

Type I, in which there is a double duct each emptying into the venous system on its respective side, has according to Davis been described by Hommel ('37), Winslow ('66), Cruickshank ('90), Sommering ('92). Such ducts were also described by Krause ('82) but Davis found no such ducts. However, Meyer ('15) found a double duct which may be classed in this type. He described it as follows:

From it (cisterna chyli) a large left thoracic duct extended cranially and emptied into the subclavian vein after being joined by the left cervical trunk. But in addition to the left thoracic duct a smaller duct ran to the right from the cisterna chyli and then extended cranially parallel to the left duct, continued directly with the left cervical and joined the left thoracic duct by a transverse branch in the bronchial region after receiving a large bronchial trunk. In addition to being double, this specimen of the thoracic duct was interesting in the fact that the right cervical trunk joined the right thoracic trunk in the retro-bronchial region and sent a transverse communicating branch which joined the left thoracic duct after receiving a large bronchial trunk.

I found no cases of this type in the 34 animals examined.

In type III, there are two thoracic ducts which terminate in the right angulus venous. In man Breschet ('36) described one case of this type but I found none in the guinea pig.

In type IV the duct is single and on the right, but bifurcates and joins the venous system on both the right and left sides. Examples of it have been described by Butler ('03), Lauth ('35), von Patruban ('44), Haller ('46), Diemerbroeck ('85) and Cousin ('98), but Davis found no such ducts in his cases and I likewise found none.

Ducts of type V, which consists of a single duct on the left which later divides and enters the venous system of the left and right sides, have not been described for the human being and I found none in the guinea pig.

Type VII, which consists of a single duct on the left which later crosses over to the right and joins the venous system on that side, like type V, likewise has never been described in man. Neither did I find any ducts of these types, nor of type VIII, which

is a single duct on the right side which enters the venous system on that side. Cases of ducts of this type have been described by Todd ('39), Watson ('72), Fife and Haller ('75), Cruickshank ('90), Fleischmann ('15) and Davis ('15).

In the guinea pig, I found only three types—2, 6 and 8—which fall in Davis' classification for man.

In comparing the thoracic duct of the guinea pig with those of other animals, there are some similarities and marked differences. In the dog the thoracic duct may be single throughout, but often divides into two branches which unite in an ampulla. The primitive plexiform arrangement persists in varying degree. In the pig the thoracic duct divides into two ducts which unite to form an ampulla. In the guinea pig in 5 cases (figs. 3, 5, 6, 17 and 30) out of my 34, the duct divided after union of the right and left and joined again before entering the venous system, but there was no well-marked ampulla as described in the dog and pig and in some human cases. In the cat there is but one duct which usually divides in the upper part of the thorax into two or three vessels, which pass along parallel and then unite before the duct enters the left external jugular vein. None of my cases in the guinea pig represent this plan except that shown in figure 23 provided however the short piece of the right thoracic duct and the thoracic plexus had been absent and the dilation above the diaphragm had been in the abdomen just below the diaphragm. In the horse according to Sisson, the thoracic duct takes quite a tortuous course. The duct in the horse is often double thus agreeing with the condition in the guinea pig. In the ox too the duct is rarely ever single, but often double and plexiform. Sala ('99) and Pensa ('08) also picture bilateral symmetrical ducts in birds. Wiedesheim ('07) gives bilateral symmetrical ducts in the chick with a plexus between the lower part and at the root of the mesentery. Schimkewitsch ('10) also pictures bilateral symmetrical ducts in the frog. Huntington ('11) says that the thoracic duct of the cat is potentially bilaterally symmetrical.

Out of the 34 cases in the guinea pig not one had any connection with the right lymphatico-venous communication. In

the 40 cases described by Parsons and Sargent in man, in 18 of the cases, or 45 per cent, the duct divided and this division usually lay behind the left common carotid artery about 5 cm. or less from its termination. The divisions were usually of the same size, but if of unequal size, the upper division was usually the larger. In 3 of these 18 cases of bifurcating ducts, he found that one divided again, forming a kind of plexus, while in 1 of the cases both divisions divided again. In 9 of the 18 cases the 2 ducts united again before reaching the vein and emptied only as one duct. In one they even joined as late as in the wall of the vein, but the others joined before. Job ('15) in 50 rats found no case in which the duct or a branch entered on the right side.

LYMPHATICO-VENOUS COMMUNICATION

Concerning lymphatico-venous communications, much more has been done and reported than on the thoracic duct as a whole. McClure and Silvester ('09) have described the communications in fifty animals covering 25 species and have pictured 31 cases. They have given 9 types of lymphatico-venous communications which greatly facilitates the classification of communications. In speaking of the common jugular district and the jugulo-subclavian districts as points of communication in a large number of cats, they say,

Neither one of these two districts predominates as the place of communication between the lymphatics and the veins, but either may serve equally in this capacity. If communications are so consistent they should rest upon a morphological interpretation of their development.

In the guinea pig, 33 out of 34 cases or 97 per cent, had single communications. The other one (fig. 6), or 2.9 per cent, had a double communication. As regards the left lymphatico-venous communications, all of my cases come under type 1, in which there are two lymphatico-venous communications on the left side. Even the case (fig. 6) in which there is a double communication belongs here, for in this case both communications are in the common jugular region. There is a predomi-

nance of single over multiple communications at the two usual districts of lymphatico-venous communications. One where the thoracic duct terminates in the common jugular district, and the other where the brachial lymphatics terminate in the jugulo-subclavian district. In the one case in which McClure and Silvester picture the left lymphatico-venous communication in the guinea pig, there is a connection between the termination of the thoracic duct and that of the cervical trunk with that of the termination of the brachial trunk. In several cases a branch from the thoracic duct near its termination, extended brachially, but in no case did I observe a communication. McClure and Silvester have pictured, in the guinea pig, a large cervical lymph node with a lymph vessel several centimeters long connecting with the thoracic duct just before its termination. I found this present in all my animals as shown in figure 3. In a large number of the cases the injected ink passed from the thoracic duct up into this cervical trunk about 2 cm. (fig. 15).

I have arranged several tables from Davis and modified them giving the percentage of the different kinds of termination in man and the guinea pig.

In table 5, the similarity between the guinea pig and man in regard to the large percentage of single terminations will be readily noticed. In the 34 cases examined, only one case (fig. 6) had more than one termination, this being double. Termi-

TABLE 1
Termination of guinea pig (34 cases)

MODE	PLACE OF TERMINATION	CASES	PER CENT	SEE FIGURES
Single.....	Left internal jugulo-sub-clavian junction	15	44.11	1, 2, 4, 5, 9, 10, 11, 12, 13, 14, 16, 17, 18, 27, 32
Single.....	Left jugular angle	13	38.23	3, 7, 15, 19, 20, 21, 22, 24, 28, 29, 30, 33, 34
Single.....	Left internal jugular	2	5.88	26, 31
Single.....	Left external jugular	3	8.82	8, 23, 25
Double.....	1 division subclavian; 1 division at internal jugular and subclavian junction	1	2.94	6

TABLE 2

Parsons and Sargent's 40 human cases. Tables 2, 3, 4, after Davis

MODE	PLACE OF TERMINATION	CASES	PER CENT
Single.....	Left internal jugular	28	70.00
Single.....	Left internal jugular	3	7.50
Double.....	Left internal jugular	4	10.25
Double.....	Left internal jugular and some other vein	2	5.00
Double.....	1 branch in left internal jugular; 1 branch in left subclavian	1	2.50
Quadruple.....	Left internal jugular	1	2.50
Quadruple.....	1 branch in left internal jugular; 3 branches in left subclavian	1	2.50

TABLE 3

Wendel's 17 human cases termination

MODE	PLACE OF TERMINATION	CASES	PER CENT
Single.....	Not stated	9	52.94
Double.....	Not stated	3	17.64
Triple.....	Not stated	1	5.88
Multiple.....	Not stated	4	23.52

Boullard's cases reported by Verneuil

Single.....	Not stated	18	74.98
Double.....	Not stated	3	12.49
Triple.....	Not stated	2	8.33
Sixfold.....	Not stated	1	4.16

TABLE 4

Davis' 22 human cases

MODE	TERMINATION	CASES	PER CENT
Single.....	Left angulus venosus	5	22.72
Single.....	Left subclavian	10	45.45
Single.....	Left internal jugular	1	4.54
Single.....	Left innominate	1	4.54
Double.....	1 branch of left internal jugular; 1 branch in angulus venosus	1	4.54
Double.....	Left subclavian	1	4.54
Triple.....	2 branches in left internal jugular; 1 branch in left vertebral	1	4.54
Triple.....	Right internal jugular	1	4.54
Quadruple	Left subclavian	1	4.54

TABLE 5
Man and guinea pig

MODE OF TERMINATION	PARSONS AND SARGENT	BOULLARD	WENDEL	DAVIS	CLATTEN- BURG GUINEA PIG
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Single	77.50	74.98	52.94	77.26	97.05
Double	17.50	12.49	17.61	9.09	2.93
Triple		8.33	5.88	9.09	
Quadruple	5.00			4.54	
Multiple			23.52		
Sixfold		4.16			

nations of the thoracic duct on the right side, both of a single and double duct, have been described by a number of authors in man. Absence of the thoracic duct in a new born child has also been reported by Smith ('89).

In the cat the termination of the duct is single at the external jugular near the jugulo-subclavian junction according to Reighard and Jennings ('01). In the dog the termination is in the left brachio-cephalic vein according to Chaveau ('10), while in the pig its termination is near that of the left jugular vein. In the horse according to Sisson ('11) the termination is in the left anterior vena cava behind the jugulars. In the frog and chick in which double ducts occur as in the guinea pig, they are said to terminate in the superior vena cavae making the ducts bilaterally symmetrical.

A number of different authors have described lymphatico-venous communications other than those at the base of the neck. Sylvester ('12) described the presence of permanent communications between the lymphatics and venous system at the level of the renal veins in the adult South American monkeys. Job ('15) also described a portal communication and an ilio-lumbar connection besides the renal lymphatico-venous communications in the common rat. Wutzer ('45) in man and Boddaert ('99) in the rabbit have described the termination of the thoracic duct in the azygos vein.

In one case (fig. 3) in the guinea pig, I found a communication which differs from any that I have yet seen described. In

this case there were the two ducts as usual which united between the first and second vertebra. However, just before this union they were connected just below the left innominate artery. Just caudal to this cross connection there was a collateral which ran to the left and cranially from the left duct. This collateral was about half a centimeter in length and connected with a vein. I verified this communication by forcing all the ink out of the collateral and the vein, and then by pressure, forced more ink out through this channel from the left thoracic duct and into the vein. Small masses that passed along could easily be seen through the thin walls of the vessels by the use of a dissecting microscope. The vein with which this communication took place corresponds, as far as concerns the area it drains, to the accessory hemi-azygos but not as to its termination. It terminated in the left innominate vein just medial to the internal jugular vein. I might mention that in the guinea pig the veins corresponding to the azygos and hemi-azygos lie dorsal to the ribs and there is no communications between them, ventral to the vertebra. Huntington ('10) in speaking of connections other than at the common jugular and jugulo-subclavian angles, says that

Such connections, if they exist in certain forms, must be interpreted with our present knowledge of mammalian lymphatic organization, as retention of other primitive lymph heart bonds between the lymphatic and venous system which, in the greater number of mammalia, are not developed and carried into the typical adult plan, but which may, while a typical for the general class, appear in certain specialized forms. It is possible that the reported instances of the termination of the thoracic duct in one of the azygos veins or its tributaries in the adult human subjects can be interpreted as variations depending for their genesis on the atypical development and retention of a lymphatico-venous heart formation at points other than the ones normally concerned in the production of the jugular lymph sac. The reported cases are, however, not sufficiently authentic to accept them at their face value, and the available evidence is too scanty to warrant the assumption that these variations, if they exist, are of a progressive character tending towards the eventual reduction of the thoracic duct and substitution for the same of a more direct connection of the abdominal lymphatic channels with the venous system.

RECEPTACULUM CHYLI

In man Davis ('15) found the cisterna chyli present in 50 per cent of his cases. The cisterna chyli being usually situated between the 12th thoracic and 2nd lumbar vertebra. In the dog the cisterna chyli is large and fusiform. In the ox the cisterna chyli is small and variable, while in the horse it is rather elongated, measuring about 10 cm. In both the cat and rabbit there is a large and well-defined receptaculum chyli. In all of the mammals mentioned above in which a cisterna chyli exists it is situated in the abdomen. In the guinea pig, I found no abdominal cisterna chyli, but in 73.52 per cent of the cases (table 6) there was a well-marked dilation in the thorax varying in location between the 11th to 13th thoracic vertebra. These dilations if they are to be called cisterna chyli must be designated as thoracic cisterna chyli. Table 6 shows the cases in which the dilations occurred and their relation to the right or left thoracic ducts.

LYMPH NODES

The nodes represented in the figures are those of the abdomino-inguinal, external iliac, mesenteric and cervical regions and those on each side of the superior lumbar plexus. In addition to these, nodes occurred in the dorsal lumbar plexus in a number of cases (figs. 1, 2, 3, etc.). Davis pictured 45 per cent of the ducts connected with thoracic lymph nodes.

According to Sabin ('12) lymph glands develop from a lymphatic plexus and Pensa ('09) states lymph glands may occur along the course of the thoracic duct. Since in the guinea pig there is such a large plexus between the lower half of the two thoracic ducts, one might expect to find lymph nodes here but in no animal were thoracic lymph nodes injected. Moreover, none were observed uninjected in relation to this plexus. In fact, the only lymph nodes noticed in the thorax were the bronchial nodes and a large node or so at the apex of the thorax. McClure and Silvester picture in guinea pigs a channel connecting one of these nodes at the apex of the thoracic cavity, and the right thoracic

TABLE 6

NUMBER	TYPE OF DUCT				CYSTIC DUCT PRESENT				INTERNAL JUG- ULAR AND IN- NOMINATE FUNCTION	INNOMINATE BETWEEN CLAVIS	INTERNAL JUGULAR	EXTERNAL JUG- ULAR INNOV- ATION	LOOP AT LEFT 6TH RIB	COMMUNICA- TION 1ST TO 2ND RIB	SEX		NUMBER OF RIBS		
	Single	Right	Left	Double	Right	Median	Left	Male							Female	13	12		
1				+										+		+	+	+	
2																			
3																			
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duct. Occasionally some of the nodes in relation with the superior lumbar plexus were double and sometimes like a figure 8 (figs. 1, 3, 6, 8, 13, and 30). Job ('15) also described such nodes in the rat. Job states that in this node the caudal part becomes injected, just as the single nodes of the head, knee, elbow and caudal region do, while the anterior part responds as the intestinal and thoracic nodes. As these double nodes were never revealed until the injection was completed and the animal fixed, I am unable to state the manner of injection in the guinea pig.

CONCLUSION

Hence it appears that the normal thoracic duct is double in the guinea pig and consists of a right and left duct lying at the respective sides of the thoracic aorta for the greater distance. The right duct passes behind the aorta at about the 5th rib, then runs cephalad and joins the left duct before terminating. The termination is generally single and terminates in the region of the junctions of the left jugulars. A thoracic dilation corresponding to a cisterna chyli occurs in 73.52 per cent of this series, just above the diaphragm, about the level of the 12th rib.

As can readily be noted from table 6, there is no apparent difference between any of the important features of the thoracic duct in the male and female. Davis in his 22 cases of man pictures collaterals in the thoracic cavity in every case. In the guinea pig collaterals in the thorax were only present in 5 out of 34 cases or in (figs. 3, 5, 16, 18 and 19), 17.6 per cent. Davis also found in man, a division of the main trunk into two trunks which unite again to form a single trunk, in 59 per cent of his cases. In the guinea pig I found one or more such instances in 25 out of the 34 cases examined or in 73.5 per cent (figs. 1, 2, 4, 13, 15, 17-20, 22-24, 25, 28, 29, 31 and 34). In connection with these 'insula' as Haller is said to have called them, it is interesting to note a loop, which comes under this designation, in 11 cases out of 34 (figs. 4, 10, 16, 18, 20, 22, 24, 25, 28, 31 and 34), at about the level of the 6th rib, and in relation with the left thoracic duct on its left side.

Before the right duct joined the left there was a connection between the two just below the left innominate artery in 61.7 per cent of the cases. In those cases in which there were 12 ribs and a cisterna chyli, the latter was usually situated a little more cephalad than in the other cases. Seven of the 34 guinea pigs, or 20.5 per cent had 12 ribs, while the other had 13 ribs.

In conclusion it affords me much pleasure to thank Professor Meyer for his valuable assistance and the suggestions which made this work possible.

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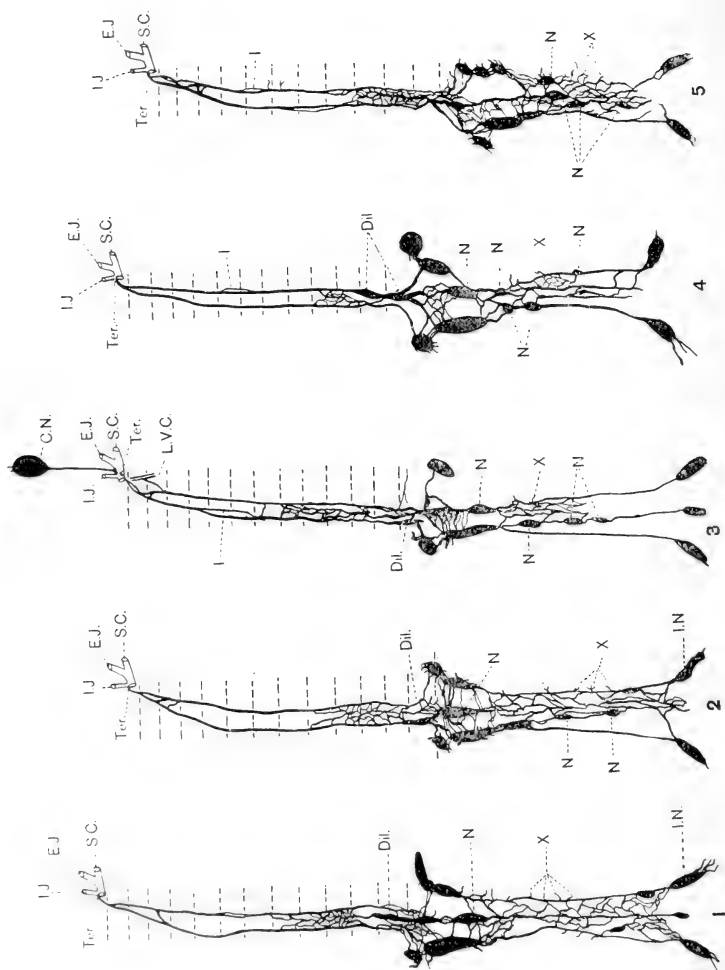
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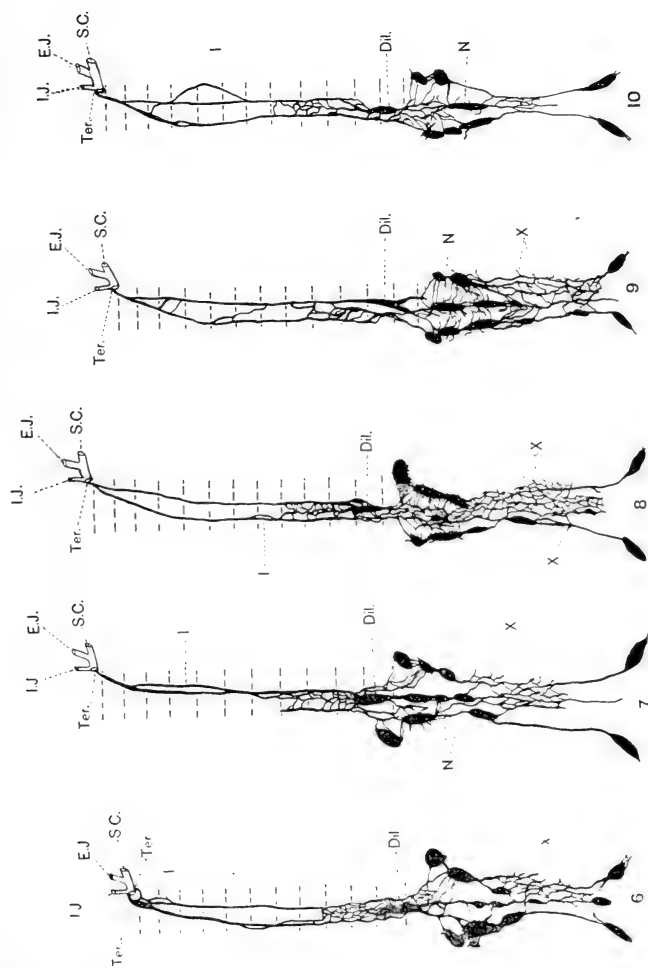
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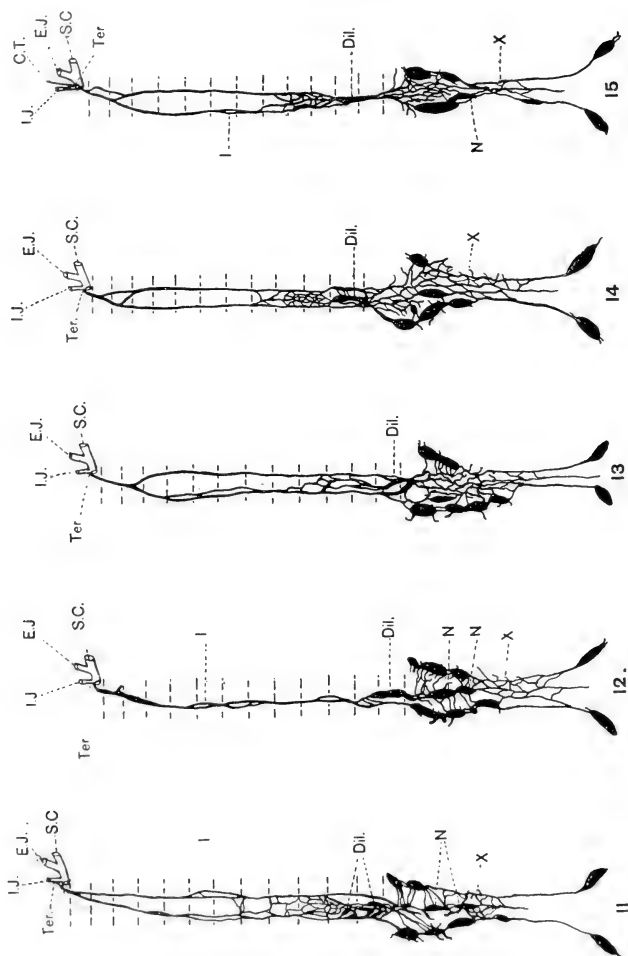
EXPLANATION OF FIGURES

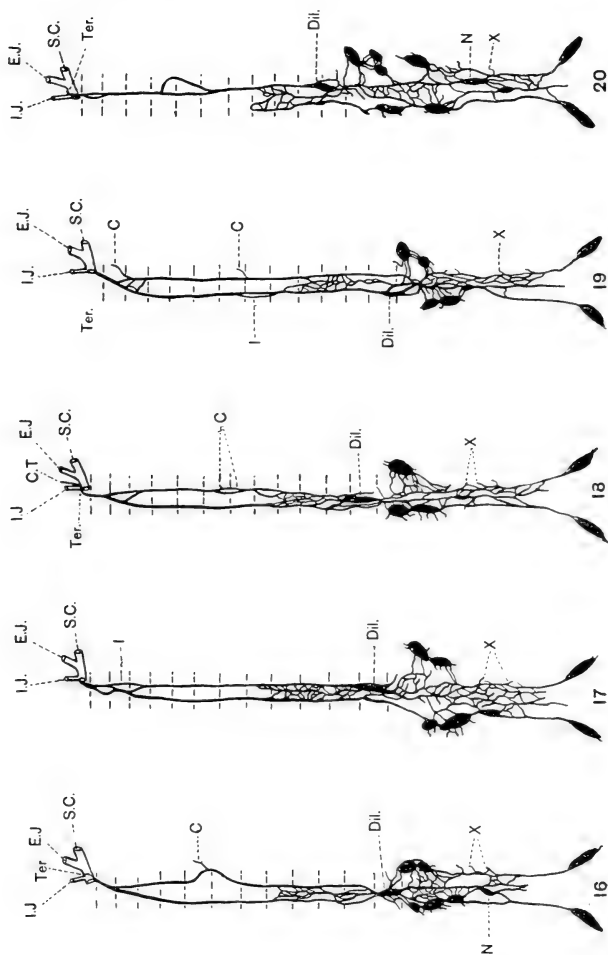
The transverse dotted lines in the figures mark the levels of the ribs. The solid black nodules, unless labelled otherwise, represent lymph nodes.

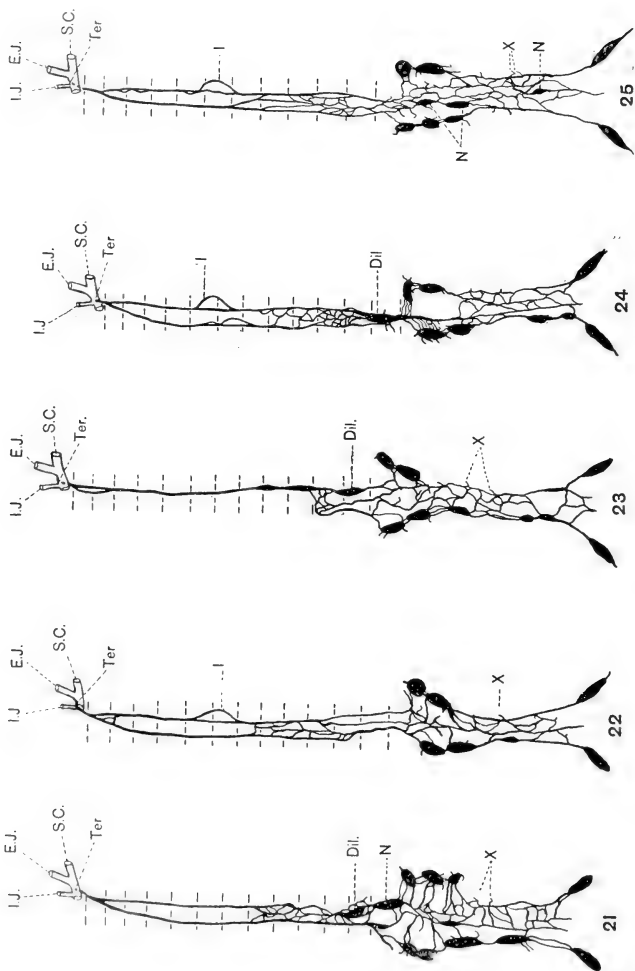
<i>I. J.</i> , internal jugular	<i>Ter.</i> , indicates termination of thoracic duct
<i>E. J.</i> , external jugular	<i>Dil.</i> , dilatation of thoracic duct
<i>S. C.</i> , sub-clavian	<i>I.</i> , insula
<i>C. T.</i> , cervical trunk	<i>C.</i> , Collateral
<i>In.</i> , innominate vein	<i>N.</i> , node
<i>X.</i> , cut ends of ventral lumbar plexus	<i>I. N.</i> , iliac node
<i>L. V. C.</i> , lymphatico-venous communication	<i>C. N.</i> , cervical node

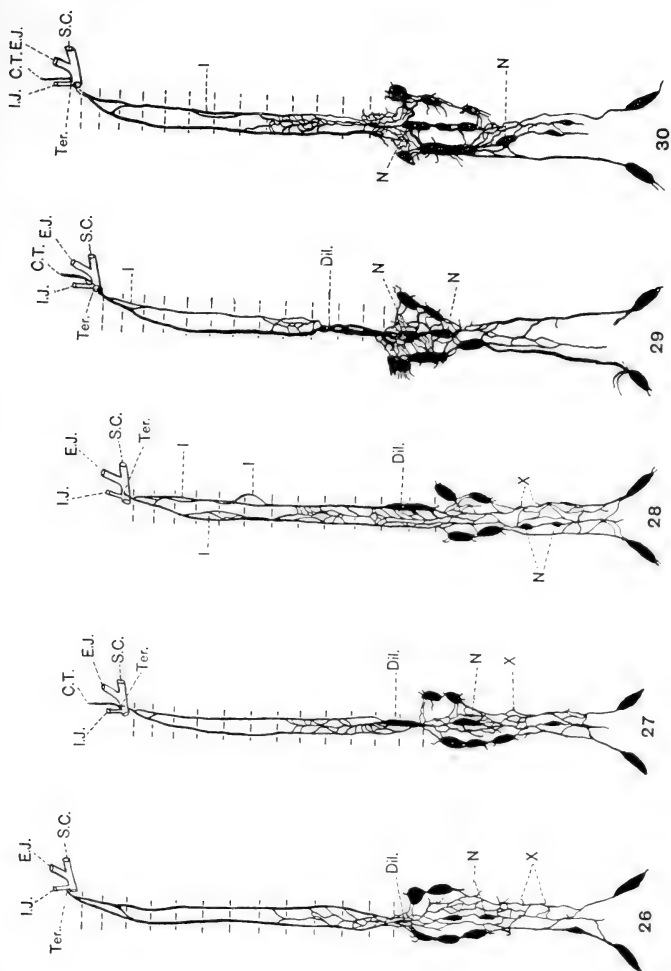


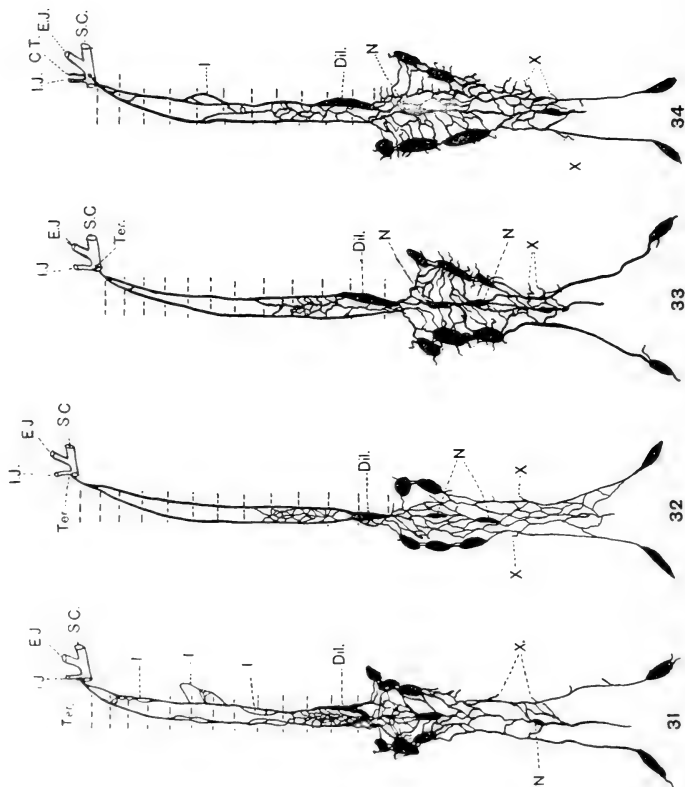












OBSERVATIONS ON THE SWEAT GLANDS OF TROPICAL AND NORTHERN RACES

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TWO FIGURES

The present preliminary report on the sweat glands was begun as a part of a joint investigation suggested by the late Paul C. Freer, Director of the Bureau of Science of Manila. The general problem was the supposed untoward effect of a tropical residence on the white man and the supposed constitutional adaptability of the dark races to a tropical climate. The investigations were undertaken in a coöperative way by chemists and physiologists of the Bureau of Science, the departments of Anatomy, Pharmacology, Physiology and Physics of the University of the Philippines and the United States Army Medical Board for the Study of Tropical Diseases.

Certain claims of Daubler ('00) and Aron ('11) seem to indicate at least one definite adaptation of the dark races to the tropics and lead us to investigate the sweat glands. Daubler states that the size of the sweat glands of the native of tropical Africa is much greater than that of the European. Aron finds that the sweating apparatus of the aborigines of the Philippines, the Negritos, is much superior to that of the white man. This superiority he says is shown by the difference in the manner of sweating rather than in the amount of sweat produced. According to Aron, the Negrito secretes small beads of sweat over the entire body, which soon forms a thin film. As the whole surface of the body is covered by this water film, the maximum cooling effect from evaporation is obtained. In the case of the white man, on the other hand, the sweating is practically limited to certain areas of the body surface. In these areas the sweating

may be quite profuse, but, as most of it drops off, comparatively little cooling effect from evaporation is produced. He suggests that the Negrito has a greater number of sweat glands which are more equally distributed over the entire body.

We have attempted to compare the sweat glands of the tropical races with those of the northern races. Our observations at present scarcely extend beyond a comparison of the number of sweat glands in certain definite skin areas of various races. Similar comparisons of other areas will be made as material is collected. A comparison as to size is an almost endless task as is shown by the work of Huber and Adamson ('03). These investigators have found, from measurements of reconstruction a great variation in the size of the sweat glands in the Caucasian. The length of the tubule in the coiled portion of the gland from the plantar region of the foot of an adult was 4.25 mm. A gland from the hairy portion of the pubic region of a woman was found to measure 10.4 mm. They have further shown, and we have confirmed their findings, that it is almost impossible to determine with any degree of accuracy the size of sweat glands without making reconstructions of them. It is even

now and then exceedingly difficult in a series of sections to trace with certainty a single gland, especially toward the beginning and end of a series of sections of any one gland. Loops of neighboring glands are often in close proximity and are apparently surrounded by a common layer of somewhat denser connective tissue so that a separation of tubules belonging to two, or now and then even three, contiguous glands can be made with certainty only by reconstruction.

In maceration preparations of skin from the palmar region and from the chest in both American and Filipino we have noted a great variation in the size of the glands in each piece of skin taken. Frequently a given gland was fully twice as large as its neighbor. It is thus apparent that a racial comparison of sweat glands as to size to be of any pretence to accuracy must be based upon measurements of a vast number of glands in different portions of the skin of the several races.

TECHNIQUE

In our study of the number and distribution of the sweat glands we find that there is less variation in those occurring on the plantar surface of the foot and the palmar surface of the hand than in other regions of the body. The sweat glands first make their appearance in the embryo in these regions. These areas also lend themselves readily to an extensive enumeration of the sweat glands. Our observations so far have been made principally on these regions. In a warm climate prints of the fingers, palms, toes and plantar surfaces of the feet can be made which in many cases will give a negative impression of every gland duct in the area printed. We have employed the method which is in use in the recruiting office of the United States Army. A very thin layer of the best printer's ink is rolled out with a small hand roller upon a glass plate. The subject's finger (hand or toe, etc.) is carefully and lightly pressed first upon the ink, and next upon a special type of glazed paper. The impressions, when made under suitable conditions, are remarkably clear and the duct of every sweat gland can usually be accounted for. Skin with much cornified epithelium will not make a satisfactory print unless pains are taken to macerate with a dilute caustic and scrape off the surface tissue.

The gland ducts may be counted directly upon the hands and feet with the aid of a good hand lens of about 12 diameters magnification. This is facilitated by first rubbing a little powdered graphite upon the surface of the skin to be examined. This method is far more tedious and has been used only as a check upon the print method. The print method is obviously not adapted to those areas of skin where hair and wrinkles occur. As the ducts of sweat glands frequently open into hair follicles the glands can not be counted by direct inspection. Here we have resorted to maceration methods and to stained sections of skin cut parallel to the surface. The latter method was used by Krause in his study of the number of sweat glands in the skin of the various regions of the body.



Fig. 1 Photograph of a finger print of the distal phalanx of the fourth finger (left) of an American white soldier. $\times 6.5$



Fig. 2 Drawing of a finger print to show orifices of sweat gland ducts. $\times 10$.

Through the courtesy of the surgeon-general's office United States Army and of the office of the chief surgeon, Philippinen Division of the United States Army we have had the opportunity of examining finger prints of 300 American white soldiers, 200

In counting sweat glands a glass slide with a graduated square (0.5 cm. each way) was placed over the print, graduated surface down, and the specimen magnified 10 diameters. In good prints, as stated above, every sweat duct can be counted (fig. 1). The graduated square was always placed over the print of the distal phalanx in counting the glands of the fingers, and over that area where the cristae cutis form a whorl or delta.

We have counted 248,998 sweat glands in $\frac{1}{4}$ square centimeter areas of 1,572 fingers and 38,736 in the palms, toes and plantar surfaces of the feet. Table 1 shows the average counts per square centimeter of skin area for the various races. Rather uniform variations have been observed in the distribution of sweat glands in the fingers. The number of glands varies in different areas of the volar surfaces of the fingers. The number is greatest near the distal ends and smallest in the immediate region of the flexion groove at the joints. As stated above, the

Average number of sweat glands per square centimeter of skin area in the various races as shown by fingers

[illegible]

number is most nearly constant in the region of the whorl or delta than in other regions. Comparatively few prints of the thumb have been examined. In all of these, however, the number of sweat glands has been distinctly lower than in any of the other fingers. Of the other fingers the second or index finger has shown the lowest average number of glands per unit of skin area, while the fourth or ring finger has shown by far the greatest number of glands. A more detailed comparison of the number of sweat glands per square centimeter of skin for the different fingers in the several races is given below in table 1. The average number of glands per square centimeter of skin area for the finger for all the races examined is 624.4.¹ The greatest number was found in prints of the fingers of Negrito children. It was found that the number of glands per unit of skin area for the hands, feet and toes bears a rather close racial relation to the number on the fingers, and in the different individuals varies directly with the number on the fingers. As regards the different races our results show a greater number of sweat glands in all the tropical than in the northern races.

Taking the American white soldier as the standard, the number of sweat glands per unit of skin area was found to be 6.83 per cent greater in the American negro soldier, 16.61 per cent greater in the Filipino soldier, 22.34 per cent greater in the Moro soldier, 26.81 per cent greater in the adult Negritos, 31.72 per cent greater in the Hindu and 69.82 per cent greater in the Negrito youths and children. Additional details of these counts will be found in table 1. The greater number of sweat glands per unit area with the Negrito youth and child is no doubt due to a corresponding difference in size of the individuals. As all the sweat glands are fully formed at birth² it is merely a

¹ This average is much lower than the estimation of earlier authors, thus—“Über die Menge der Knäueldrüsen haben wir ältere Angaben von Krause senior denen zufolge ihre Zahl zwischen 400-600 (Rücken, Wange, erste zwei Abschnitte der unteren Extremitäten) und 2600-2736 auf I □² Haut schwankt und die grösseren Zahlen an der hanffläche und Fusssohle sich finden. Neure Zählungen von Hörschelmann ergeben viel näher stehende Grenzzahlen von 641 Fussrucken, und IIII (vola manus) auf I □ cm und viel mehr drüsen.”—Koelliker.

² At the fourth month according to Wilder ('16).

question of the increase in skin area during growth bringing about a dispersion of the glands. The ratio of the number of sweat glands in the American white soldier (100 per cent) to that of the Filipino soldier (116.93 per cent) and the Negrito adult (124.05 per cent) shows a wider variation than the differences in size³ of the individuals of these respective groups. The American negro soldier is of the same approximate size as the American white soldier, and there are 6.83 per cent more sweat glands per unit of skin area in the former. The Hindus examined were of a larger average size⁴ than the American soldiers and showed the highest sweat gland count for adults (131.76 per cent). Thus racial variation in size does not account for the difference in ratio of the sweat glands per unit of skin area.

The number of sweat glands was determined in a similar manner from prints of the palmar surface of the hand and the plantar surface of the feet of Americans (white) and Filipinos. Successful prints were made from these areas of 6 American university men and 6 Filipino students; 325 separate areas were counted, giving a total of 38,736 glands. The average number of sweat glands on the palmar surface was 438.0 per square centimeter in the American, and 473.6 in the Filipino. On the plantar surface of the feet there were 436.4 glands per square centimeter in the American and 498.4 in the Filipino. On the plantar surfaces of the toes there was an average of 527 and 525.5 sweat glands per square centimeter in the American and Filipino respectively. Thus the number of sweat glands in the Filipino was in this series 8.1 per cent greater on the palm and 14.1 per cent greater on the plantar surface of the feet than in corresponding areas in the American. In all these counts there was very little individual variation. Different individuals of the same group gave almost the same count.

³ Captain Davis of the recruiting office of the United States Army in Manila tells us the average weight of the American white soldier is approximately 150 pounds and of the Filipino scout approximately 130 pounds. The Negritos are smaller and can be estimated at about 120 pounds.

⁴ All these Hindoos were tall, large and portly and averaged 160-165 pounds in weight.

We are not able to confirm Aron's observation that the tropical aborigines secrete only small beads of sweat over the entire body. On two tramping expeditions in the mountains of the Philippines which we were fortunate enough to arrange with a number of Negritos we observed streams of sweat running down the back, and copious sweating on scalp, forehead and face and sweat dripping from the chin. When making finger prints in camp it was necessary repeatedly to dry off droplets of sweat from the fingers of the Negritos.

From the few maceration preparations mentioned above we are not able to discern any difference in the size of the sweat glands of the American and the Filipino.

As regards number, all our observations show a higher count in all the tropical races. These counts, furthermore, were made on those areas in which the number of sweat glands is the most nearly constant.

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THE RELATIONS OF THE SUPERFICIAL AND DEEP LOBES OF THE PAROTID GLAND TO THE DUCTS AND TO THE FACIAL NERVE

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TWO FIGURES

The parotid gland is described by the current English, French and German text-books of anatomy as essentially a single mass, with various projections, occupying the retro-mandibular fossa and perforated by the facial nerve and its branches.

According to Grégoire ('12), Luschka recognized the division of the parotid into two portions. He quotes Luschka as follows: "Le nerf facial et ses rameaux divisent incomplètement la gland en deux portions, l'une interne plus petite, l'autre externe plus volumineuse." Grégoire cites no reference for this statement, however, nor could I find any mention of it in Luschka's text-book of anatomy ('62 and '67).

Henle ('73), however, in describing the parotid states: "Durch den Stamm und die Hauptverästelungen des N. facialis wird sie unvollkommen in eine mächtigere äussere und eine schwächere innere Schichte abgetheilt." A similar brief statement appears in the more recent edition by Henle-Merkel ('01).

This relation was confirmed by Grégoire upon careful dissections in the human (adult and fetal) and some of the mammalian species, the monkey and the rabbit. In the guinea pig and (usually) in the dog he found the facial nerve lying entirely beneath the parotid gland. In all of these cases Grégoire described the parotid as being divided into a superficial and a deep lobe with the facial nerve and its branches lying between them, except in the dog and guinea pig. He asserted that the two parotid lobes are united at their upper extremities and that this relation results from the mode of development of the gland.

In a human fetus of three months (8 cm.) he observed the parotid gland to be entirely superficial to the facial nerve. But he found the beginning of the deep lobe in a fetus of six or seven months, and concluded that further growth of the gland upward is prevented on reaching the base of the skull and that the growing extremity is apparently deflected inward and downward internal to the facial nerve to form the deep lobe.

Corresponding to this mode of origin Grégoire described the duct from the deep lobe as passing upward and outward, above the branches of the facial nerve to unite with the duct from the superficial lobe.

Since the topography of the parotid gland and the facial nerve is of great importance (especially in surgery), a further investigation of this region was undertaken at the suggestion of Prof. C. M. Jackson, to whom, as well as to Prof. R. E. Scammon, I am indebted for assistance.

I have made careful dissections of sixty-six adult human parotids (from thirty-nine male cadavers), and of thirteen parotids in the human fetus and newborn (total length 36 cm. to 54 cm.). The more important results will be stated briefly.

The human parotid gland (late fetal and adult) consists of a large superficial lobe and a smaller deep lobe (figs. 1 and 2), which are usually readily separable with the exception of a small isthmus where they are more intimately connected.

The connecting isthmus is somewhat variable in size and position, and rarely absent. It is not at the upper extremity of the gland (where a union of the two lobes is described by Grégoire), but somewhat lower, usually located near the middle or the junction of the middle and upper thirds of the gland, and somewhat posteriorly. The isthmus, except in eight of the sixty-six adult cases, consists of gland parenchyma, together with connective tissue, including vessels, and ducts of variable size and number.

The ducts of the parotid are exceedingly variable in their relations and do not conform to the type described by Grégoire. The main parotid (Stenson's) duct may be more closely associated with the superficial lobe (31 of 64 adult cases observed),

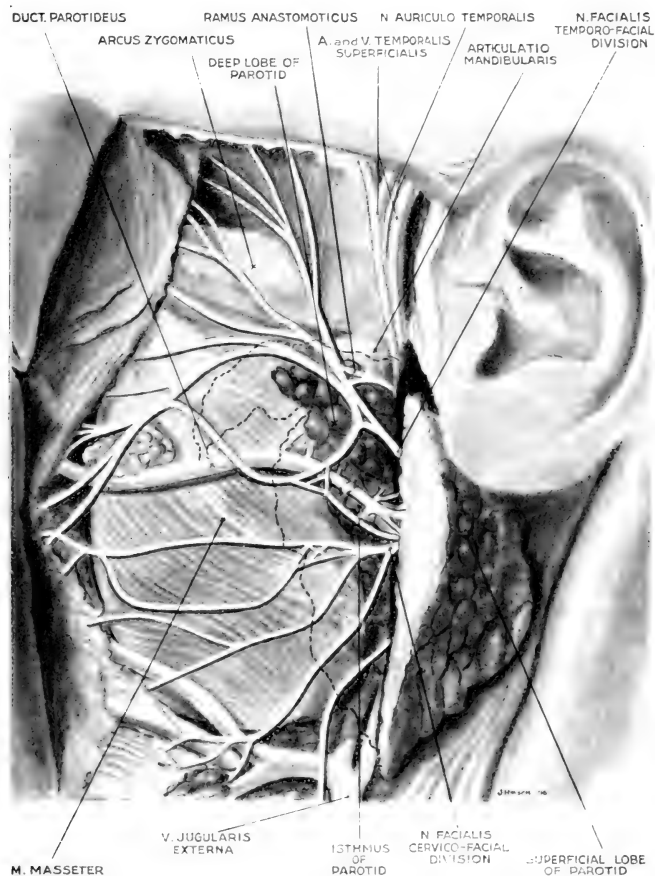


Fig. 1 Lateral view of parotid region dissected to show the relations of the superficial and the deep lobe of the parotid to the duct and to the facial nerve. The anterior portion of the superficial lobe has been removed. Its original outline is indicated by the dotted line. A small accessory lobe is shown on the anterior part of the parotid duct.

or with the deep lobe (16 of 64 cases), or may proceed between the lobes toward the region of the isthmus without intimate relation to either lobe. The main duct branches at a variable distance from the anterior border of the gland, and frequently not until reaching the region of the isthmus. The duct branches

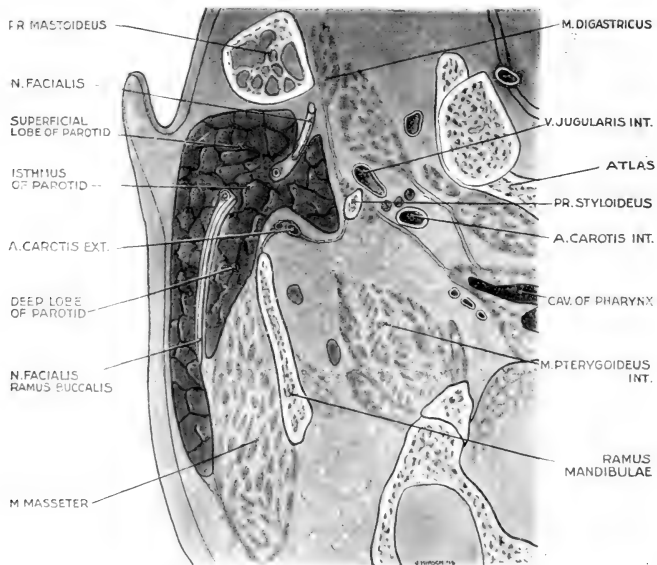


Fig. 2 Horizontal section of the head in the parotid region (semidiagrammatic) to show the relations of the superficial and the deep lobe of the parotid. The two lobes are shown separated by branches of the facial nerve, except in the region of the isthmus.

in a variable manner. Each lobe has usually a main terminal duct, which, however, sometimes receives minor branches from the other lobe. A duct draining part (or exceptionally all) of either the superficial or the deep lobe may cross the isthmus to join the duct of the opposite lobe. Small branches from either

(especially the superficial) lobe may also join the main parotid (Stenson's) duct (fig. 1).

The facial nerve trunk and its main branches lie between the superficial and the deep lobe of the parotid gland (figs. 1 and 2). These lobes are not united at their upper extremities (Λ -shaped), however, with all the nerve branches below the junction of the lobes, as described by Grégoire. The two lobes are united rather in H-shape, the connecting isthmus corresponding to the cross-bar (fig. 2). Upon approaching the isthmus of the gland from behind, the facial nerve divides into its upper (temporo-facial) division, which passes forward above the isthmus, and its lower (cervico-facial) division, which passes below the isthmus (fig. 1). The uppermost and lowermost facial branches may not come into contact with the deep lobe (on account of its small size), though still under cover of the superficial lobe of the parotid. The facial branches lie between the two lobes, sometimes infolded in grooves on the opposing surfaces. Rarely are they entirely surrounded by the gland parenchyma. In the neighborhood of the ducts, the nerve branches lie superficial to those ducts more closely associated with the deep lobe and beneath (internal to) those ducts more closely associated with the superficial lobe. Thus the relations of nerves and ducts are quite variable; but in no case were all of the ducts from the deep lobe observed to pass upward and outward, above all the facial nerve branches, as described by Grégoire. The relations found are therefore not in agreement with Grégoire's theory of the development of the parotid lobes.

In general, since the relations found in the dissections of the late fetus and newborn were essentially similar to those above mentioned for the adult, no separate description of the former is necessary. In one full term fetus of 50 cm., the two lobes were nearly equal in size, but the deep lobe was usually found to be much the smaller. It should be noted, however, that no observations were made upon younger fetuses, to determine the developmental relations.

In conclusion, the results of my investigation may be summarized briefly, as follows: The human parotid gland (late

fetal, newborn and adult) consists of a larger superficial and a smaller deep lobe, usually distinct and readily separable, with separate ducts. The facial nerve and branches lie between these lobes. The lobes are usually joined (not at their upper ends but lower down) by an isthmus separating the temporo-facial and the cervico-facial divisions of the facial nerve. The relations found are not in agreement with Grégoire's theory of the development of the parotid lobes.

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FURTHER OBSERVATIONS ON TAILLESSNESS IN THE RAT

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THREE FIGURES

This paper has three objects: first, to describe skeletal conditions in the region posterior to the lumbar vertebrae of two tailless rats which have appeared in the colony since the writing of the preceding paper (Conrow '15, *Anat. Rec.*, vol. 9, no. 10, p. 777); second, to describe skeletal conditions in the same region of four rats whose tails were removed by operation soon after birth; and third, to emphasize, by a comparison of the skeletal conditions in these two sets of rats, the conclusion of the previous paper, namely, that taillessness in the rat is due not to accident after birth but to a congenital defect in the vertebral column.

Since the writing of the previous paper (Conrow '15) four tailless rats have appeared among the standard rats (*Mus norvegicus albinus*) of the Wistar Institute Colony, making nine tailless specimens observed during the past ten years in a total of 71,500 rats. Of these four, two are at present mated in the colony, and two (a male and a female) have been examined and will be described here. The data concerning these latter are presented in table 1.

In rat No. 4, a male, the vertebrae and pelvic girdle were studied. All vertebrae caudal to the second sacral were lacking, as was a large part of the second sacral. The first sacral was somewhat deformed and had what remained of the second fused with it. Its points of attachment to the pelvic girdle were a little more caudal than usual. (Two of the cervical vertebrae also were partly fused.) The striking feature in this rat was that

TABLE 1

Data on tailless rats

RAT NO.	SPECIES	SEX	AGE	BODY WEIGHT	BODY LENGTH	DATE OF KILLING
			<i>days</i>	<i>grams</i>	<i>mm.</i>	
4	<i>Mus norvegicus albinus</i> . Standard strain.	M.	about 244	144.4 (wet)	not taken	September 13, 1915
5	<i>Mus norvegicus albinus</i> . Standard strain.	F.	361	133.8	185	August 3, 1916

the vertebral column terminated far from the posterior end of the body, even anterior to the middle of the long axis of the pelvic girdle.

In rat No. 5, a female, the four sacral vertebrae were present but they were more crowded together and fused than is usually the case. There was also one deformed cauda vertebra; the rest were lacking. This rat might not be called strictly tailless,

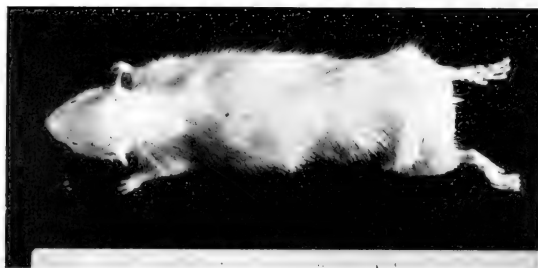


Fig. 1 Congenitally tailless rat, No. 5

since tailless, as previously defined, means "with no caudal vertebrae," but the vertebral column ended several millimeters anterior to the posterior ends of the pelvic girdle. There was an odd, external structure found here just dorsal to the anus, in the position which normally would have been held by the tail. It was a fleshy papilla 11 mm. long and 2.5 mm. in diameter, with the characteristic scaly tail covering. This structure may be seen in figure 1.

The conditions found in these two tailless rats are then (with the exception of the fleshy papilla just mentioned) similar to those found in the tailless rats previously studied.

The experiment of removing by operation the tails of several rats was conducted as follows. Two litters of standard albino new born rats were chosen. In one litter the tails were cut off as close as possible to the origin. In the other litter a slender silk thread was tightly tied about the tail of each rat at the same place. The tails of the first litter healed quickly; the tails of the second litter dropped off in a few days with apparently no inconvenience to the animals. Both litters grew normally and their eyes opened at the usual time—at the end of fifteen days. All were allowed to live, and when nearly a year old, four animals (a male and a female from each litter) were killed and examined. The data concerning them are presented in table 2.

TABLE 2
Data on rats tailless by operation

RAT NO.	SPECIES	SEX	AGE	BODY WEIGHT	BODY LENGTH	DATE OF KILLING
			days	grams	mm.	
Tails cut { 1	Mus norvegicus	M.	271	161.8	not taken	April 20, 1916
2	albinus. Standard strain	F.	289	167.4	195	May 8, 1916
Tails tied { 1	Mus norvegicus	M.	310	288.0	220	May 1, 1916
2	albinus. Standard strain	F.	317	194.2	197	May 8, 1916

Only changes in the caudal vertebrae need be mentioned here since all the sacral vertebrae of the four rats were present and normal.

Of the two rats with tails cut, rat No. 1, a male, had the first four caudal vertebrae and about half of the fifth remaining. Rat No. 2, a female, had the first three caudal vertebrae and about half of the fourth remaining. The appearance of the most posterior vertebrae of this rat and their relation to the pelvic girdle may be seen in figure 2.

Of the two rats with tails tied, rat No. 1, a male, had the first four and about half of the fifth caudal vertebra; while rat

No. 2, a female, also had the first four and a part of the fifth caudal vertebra. The appearance of the most posterior vertebrae of this last rat and their relation to the pelvic girdle may be seen in figure 3.

In each of these four rats the vertebral column extended to the posterior end of the body and all of the caudal vertebrae present were normal, except the fraction of the vertebra actually cut or tied and this showed only slight deformity.

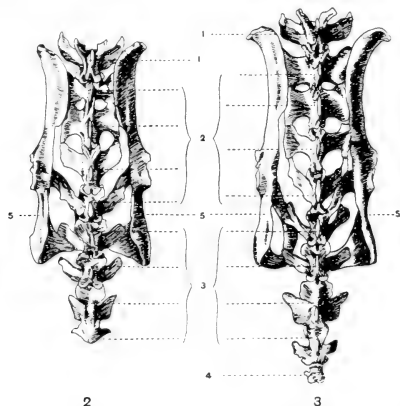


Fig. 2 Tail cut rat No. 2, dorsal aspect; 1, sixth lumbar vertebra; 2, four sacral vertebrae; 3, first four caudal vertebrae; 5, pelvic girdle.

Fig. 3 Tail tied rat No. 2, dorsal aspect; 1, sixth lumbar vertebra; 2, four sacral vertebrae; 3, first four caudal vertebrae; 4, fifth caudal vertebra; 5, pelvic girdle.

A comparison of the vertebral conditions in the congenitally tailless rats and in those rendered tailless by operation gave a marked contrast. In the former the vertebral column ended in the pelvic region, far anterior to the posterior end of the body, and also the terminal vertebrae were more or less deformed and fused; in the latter the vertebral column extended to the posterior end of the body and the vertebrae retained their normal size and shape, except those cut or tied, and even they showed only slight modification.

Since in the experiment the tails were severed as close to the body as possible, and since the vertebrae anterior to the severance not only did not disappear but kept their normal form, it is apparent that the removal of a rat's tail by operation soon after birth does not cause the disappearance of any of the remaining vertebrae, nor does it give rise to deformity or fusion among them. In other words it does not produce such a condition of the vertebrae as is found among rats born tailless, therefore taillessness in the rat as defined is due not to accident but to a congenital defect of the vertebral column.

While this paper was passing through the press, one of the two rats spoken of (p. 155) as mated in the Wistar Institute colony was killed and examined, and also two new tailless rats appeared in the colony, making a total of eleven tailless rats observed to date. The rat examined was killed on December 14, 1916. It was a female of standard strain (*Mus norvegicus albinus*); its age was 273 days, body weight 168.5 grams, and body length 193 mm. The four sacral vertebrae were present, the third and fourth being slightly deformed. There was also one deformed caudal vertebra and the rudiment of a second. The vertebral column ended far anterior to the posterior ends of the pelvic girdle. The other mated rat (a male) that was not examined is of the inbred albino strain, the 22nd generation.

The two new tailless rats (a female and a male) appeared in Dr. King's strain of small-eyed rats and the left eye of the female is small. The vertebral columns of these three rats all end anterior to the posterior ends of the pelvic girdle, for this condition may be felt distinctly by pressing the finger on the rats' backs in the pelvic girdle region.

THE SIGNIFICANCE OF THE LUNULA OF THE NAIL

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TWO FIGURES

A structure, the significance of which has always been described by a series of different answers in modern text-books of anatomy is the nail lunula. This is a small sharply circumscribed opaque area which is visible near the root of the nail of many individuals. Toldt assumed that the greater opacity of this region is due to the fact that the nail is covered here by a layer of matrix, the cells of which are undergoing active division. Unna expressed the opinion that the opacity is conditioned by the existence in this region of keratohyaline (onychogenic substance). Ranvier has given a similar explanation (quoted from Poirier and Charpy, *Traité d'anat. hum.*, Paris, 1901). He ascribed it to the existence of onychogenic granules which fill the cells of the rete mucosum. V. Brunn, on the other hand, ascribed it to the fibrous structure of the cells undergoing keratinisation in this region of the nail.

Although these particular explanations have for a long time appeared to be inadequate, it is of interest that no one, so far as the author has been able to determine, has attempted to study more carefully the peculiarities in the structure of this region. The fact that the lunula is marked off sharply from the remaining portion of the nail is a direct argument against several of the above explanations. Again, the large number of nails which one sees removed in the dispensary do not show that portion of the nail corresponding to the lunula more opaque than surrounding parts.

During the last few months a careful study of the structure of the nail and its environment has been undertaken for the

purpose of establishing, if possible, some of those conditions which regulate its continuous growth throughout the life of the individual. This study has been planned to include not only a careful investigation of the morphology, histology, and the development of the nail but also a detailed study of its connective support and vascular supply as far as they apply to the solution of the problem in question. It represents a continuation of those studies of the mechanism of cellular growth which have been carried on for some time in the laboratory by means of the tissue culture method.

It was during the course of the early part of this study that the author noticed certain peculiarities in the structure of the nail in the region of the lunula which have appeared to be important in conditioning its opacity. Since the lunula itself has a certain clinical as well as a morphological importance, and since these particular structural peculiarities have not hitherto been emphasized as important in the anatomy of any part of the body, although probably very significant as a part of the general mechanism of the growth of this structure, it became of interest to report them in a separate communication.

The nails of the hands of five individuals have now been studied. They have included those of two adult white men, an adult negro woman and two white children. Lunulae were noted at the base of uncovered portion of the body of the nails of all the fingers of the white men and on the thumbs of the children. None was seen on any of the fingers of the negro woman before the epitrichium had been removed. When the epitrichial layer is lifted, however, the sharply defined opaque lunular zone is seen. It is present in all the fingers of this individual.

A number of these fingers were dissected at once. Others were fixed in formalin, dehydrated and decalcified. The blood vessels of a number of the fingers were injected with India ink. These fingers were likewise fixed in formalin, dehydrated and decalcified. The fingers which had been fixed were either embedded in colloidin, sectioned and stained or they were sectioned free hand, examined at once and later after they had been cleared. All the unfixed fingers were hemisected along the

median sagittal plane. This was accomplished by first cutting through the nail and the soft parts with a sharp knife, the bone being later cut through with a fine saw.

In every one of these fingers examined it has been noticed that the matrix of the nail underlying the lunula is not firmly adherent to the connective tissue stratum. Throughout the whole lunula area, the two layers do not apparently adhere but lie only in contact with each other. The slightest distortion of a sagittal section of the finger leads to the separation of these two layers. The open space thus formed is sharply circumscribed. It extends from the tip of the root of the nail, at which point the matrix is adherent, to a line which corresponds to the distal margin of the lunula zone. Beyond this line and over the whole body of the nail the matrix is firmly adherent to the underlying connective tissue layer (figs. 1 and 2).

The connective tissue underneath the lunula is peculiar in structure. The fibrils near its outer edge run parallel to the lower margin of the nail matrix. They form a dense boundary layer or sheath. The connective tissue here is not vascular. The capillaries are reduced largely to a single or double layer which lies close to, and on the surface of this layer of connective tissue. The underlying portions of the connective tissue contains few capillaries.

The matrix is also peculiar in this region. Its lower margin is in most parts straight. One sees very few indentations or papillary extensions into the underlying connective tissue. This is in sharp contrast to the outer pink portion of the nail body. Here the matrix is indented both longitudinally and transversely by connective tissue papillae. The connective tissue in this region is also strikingly different from that of the lunula. It contains many vertically placed fibrils which end directly at the edge of the epithelial cells of the matrix. They adhere closely to these cells or a basement membrane. The papillae as well as the neighboring portions of the connective tissue contain large numbers of capillaries. They form a dense network which fill the papillae and neighboring parts of the connective tissue. This dense capillary network is continuous with that

of the lunula portion, the change from one form into the other taking place gradually under the distal margin of the lunula.

A number of the nails of the fingers examined have been removed by stripping them back from their outer tip. By this procedure it is possible to remove the nail with the matrix of the



Fig. 1 A thick sagittal section of the finger of a white man. A piece of paper has been passed through the opening between the matrix and the connective tissue.

Fig. 2 A thick sagittal section of a finger of a white man which has been fixed, dehydrated and decalcified. The open space between the matrix and the connective tissue layer is readily discernible in the area corresponding to the lunula.

lunula attached to it. Throughout the body of the nail the matrix is invariably torn. In none of the nails thus removed has the lunular portion been found to be more opaque than the remaining portions. Quite the reverse, this area is frequently more translucent. In a number of the nails which had been

previously fixed, dehydrated and decalcified, the tip of the root does show a definite opacity. In none of these cases, however, is this opacity seen as far forward as the distal margin of the lunular area. It fades and gradually disappears a short distance distal to the tip of the root. Furthermore, this opacity is not noticed in all the fixed specimens.

From these latter observations it seems evident that the opacity of the lunula is not conditioned by any peculiarity of the structure of the nail itself nor its matrix. Moreover, that the decrease in the capillary bed of the lunular area is not wholly, if at all, responsible for the opacity may be deduced from the fact that its change in density at the distal margin of the lunula is never as abrupt as the boundary line of the lunula demands. Further proof against this peculiarity playing any important rôle in conditioning the opacity is given by comparing the appearance of the lunular area with a portion of the body from which the blood has been removed by pressure. The lunula has a definite opacity, while that portion of the body from which the blood has been removed has a greyish translucency.

In the absence of other possibilities, the author has been led to believe, therefore, that the lunular opacity is the result of a reflection of the light at the surfaces of the junction of the matrix and the connective tissue of this portion of the nail. In the outer portion of the nail where the matrix adheres closely to the underlying connective tissue the light is transmitted directly to the capillary bed giving it its characteristic pink color; while in the region of the lunula the well formed non-adherent surfaces of both the connective tissue and the matrix reflect the light. More direct proof of this assertion may be readily obtained by pulling a portion of the body of a living nail loose from the connective tissue, thus forming such a surface in this region. During the past few weeks the author has been performing work which has led to the production of this injury. In each instance when it has occurred the detached portion has shown an opacity quite indistinguishable from the opacity of the lunula of the same finger. It is of interest, however, that unless the injury is extensive the nail will again after a few hours become adherent and assume its former pink color.

The decrease in the capillary bed of the lunular area has been described (Poirier and Charpy, *Traité d'anat. hum.*, Paris, 1901). No one, on the other hand, as far as the author has been able to determine, has described the peculiarity of adhesion between the matrix and the connective tissue in this region, nor the relation of this peculiarity in structure to the lunular opacity. It will be of further interest to ascertain whether many of the pathological opacities of the nail are not the result of similar changes, and to study more carefully the conditions which lead to the adhesion of the matrix in outer portion as contrasted with that of the lunular zone, and the general changes in the connective tissue and in the arrangement and density of the capillaries in these two regions. The nail is one of those peculiar parts of the body which continues to grow throughout the whole life of the body. A more careful investigation of its general structure and the chemical and physical changes which accompany its growth may lead to many facts of importance for ultimately ascertaining those conditions which bring about and regulate the growth of other body structures.

A PRELIMINARY EXPERIMENTAL STUDY ON THE RELATION BETWEEN MITOCHONDRIA AND DISCHARGE OF NERVOUS ACTIVITY

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We have heard a great deal about mitochondria in nerve cells, but unfortunately their study has not emerged from the purely descriptive into the experimental stage. The only record of experimental work on mitochondria in the nervous system is a brief note by Luna ('13, p. 415) on the changes in mitochondria in nerve cells following section of their peripheral processes. He found that the first changes consisted in a loss of the regular distribution of mitochondria, in an increase in their volume and in an increase in their affinity for iron hematoxylin; while in the more advanced stages of degeneration the mitochondria disappeared completely. Archimede Busacca ('15, p. 232), working on the eye, found that mitochondria in the nerve cells of the retina increased in number on light stimulation. Accordingly it is a very pertinent question to inquire whether there are alterations in mitochondria of nerve cells in muscular fatigue.

White mice were selected for the experiments because they are the smallest mammals which can be conveniently used in the laboratory and on account of the fact that more is known of the quantitative (Thurlow '16) and qualitative (Nicholson '16) variations in mitochondria in their nervous system than in that of any other animal.

They were fatigued by the very simple method, which Prof. Tamao Saito uses, of letting them swim in water until they are exhausted. It was soon discovered that they swim better when the water is slightly agitated and is raised to body temperature. Otherwise they soon learn to float and refuse to exercise.

The experiments were controlled in the usual manner by using only mice of known age and by examining, in exactly the same way, an unexercised mouse of the same litter for comparison. Five experiments of this kind were made, the mice swimming from one to two hours continuously before they were completely exhausted. (Larger mammals will swim for a day or more before exhaustion). Experience showed that young mice twenty-five to thirty days old are more suitable than adults because they are more easily tired.

In each of the five experiments the fatigued mouse and the control mouse from the same litter were chloroformed. They were then fixed by the injection of a formalin and bichromate mixture in accordance with the method advised by Cowdry ('16, p. 34). The brains were removed, mordanted, washed, dehydrated and cleared together in the same bottle. They were embedded in the same block of paraffin. Sections, cut $4\ \mu$ in thickness from the fatigued and from the control, were mounted on the same slide so as to avoid variations in the staining which might otherwise occur. The sections were then stained with fuchsin and methyl green, the fuchsin coloring the mitochondria crimson and the methyl green staining the Nissl substance a bluish green color. Preparations were made in this way of the cortex, the cerebellum and the spinal cord from each of the five experiments.

The mitochondria show a slight numerical increase in the fatigued animals in three of the experiments, but the other two experiments do not show it, indeed, in one of them there is a definite decrease in the number of the mitochondria; in any case, the variations in the number of mitochondria fall well within the range of variation which was found to be normal for the species.

Neither could any definite change in the form of mitochondria be seen in the fatigued animals. True, in the Purkinje cells of the cerebellum some of the mitochondria were swollen up to form spherules ($0.5\ \mu$ to $1\ \mu$ in diameter), but, on careful examination some of the controls showed examples of the same phenomenon, though in less marked degree.

There was a tendency toward a clumping of the mitochondria in the cytoplasm of the fatigued animals as contrasted with the more or less uniform distribution in corresponding nerve cells of the control. This clumping, when it occurs, is particularly apparent in Purkinje cells at the base of the large dendrite in close relation with the 'capuchon chromatique' of Cajal.

The Nissl substance is well fixed and is stained brightly with methyl green by the technique employed. A mild degree of chromatolysis was noticed in all the fatigued animals, in variable amount. It was usually most marked in the Purkinje cells of the cerebellum and in the pyramidal cells of the cortex. This would be inclined to lead one astray were it not for the fact that one of the normal control animals showed just as extensive chromatolysis if not more extensive than any of the fatigued ones.

Vacuoles and clear, tortuous, unstained canals (the canalicular apparatus of authors) were of common occurrence in both the fatigued and the control brains. In two of the experiments they were certainly wider and more clearly cut in the anterior horn cells of the fatigued than in those of the normal, but, again, this difference was not apparent in the other three.

The nuclei and nucleoli were given careful attention and proved entirely negative as far as a definite change is concerned in the fatigued animals. Unfortunately preparations were not made by the neurofibrillar methods and by the positive methods for the demonstration of the Golgi apparatus.

Chromophile cells could be seen in both the fatigued and the control brains. They were never observed except in the higher centers, that is, the cerebral cortex and the cerebellum. The chromophile cells stained uniformly red with fuchsin (Cowdry '16, p. 36). If anything, they were slightly increased in number in the fatigued animals.

It is evident, therefore, that the net result of these five experiments, with their controls, is to show that the mitochondria are surprisingly constant in nerve cells, rather more so even than the Nissl substance which is supposed to be concerned with the activity of nerve cells 'per se.' A fair degree of fa-

tigue, as well as a certain amount of fright, brings about no constant changes in them. It is certainly no longer necessary to take elaborate precautions in composing the minds of animals before killing them for studies on the mitochondria in the central nervous system. It is quite possible that more prolonged exhaustion will lead to definite and precise alterations in mitochondria in nerve cells. But it would appear that the study of mitochondria in fatigue in muscle cells, thrown into isometric tetanus outside the body, is more likely to bring about changes in them.

Mitochondria occur in all nerve cells though we have very little idea of what their true significance is. Certain it is, however, that they are stable structures which are not essentially altered with slight fatigue. They are not associated with the specific activity of the nervous system, that is to say with the explosive discharge of the nervous impulse, any more than they are concerned with the elaboration of secretion in pancreas cells. Key ('16, p. 216) found that the mitochondria are not exhausted in the acinus cells of the pancreas by even prolonged stimulation with secretion and pilocarpin.

This independence of mitochondria of the specialized activities of cells is in general accordance with the views which have been expressed by many authors to the effect that they are concerned with the basic fundamental processes of metabolism (some hold with oxidations) which all cells possess in common. In fact this is the conclusion to which Cowdry ('14, p. 17) has arrived in the case of the nerve cell.

It also falls in line with the generally recognized fact that with starvation and other influences which bring about changes in metabolism, the nervous system is more difficult to alter than any other tissue in the body; as well as with the unsatisfactory results which have usually attended attempts to alter the metabolism of the nervous system through excessive mental work and other agencies. I refer to the calorimeter experiments of Benedict.

Furthermore, these processes of metabolism are, as one would naturally expect, very easily modified in pathological states;

hence the sensitivity of mitochondria to pathological change. Scott ('16, p. 250) discovered that the mitochondria are the first of all the cell constituents of the pancreas to become altered in experimental phosphorus poisoning. Moreover, the fact, which is now emerging from the numerous recent pathological studies on mitochondria, that mitochondria in different types of cells respond in much the same way to different varieties of injurious influences, in other words, that there is nothing specific in the reactions of mitochondria to pathological change, is also in accord with the prevalent conception that they take part in a type of activity common to many cells.

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AN ANOMALOUS VENA PULMONALIS WITHIN THE LUNG

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TWO FIGURES

Anomalies in the distribution of the Venae pulmonales within the lung, so far as the author has been able to discover, have not been described. The only cases which he has been able to find were extra-pulmonary, and were either variations in the fusions of the venous trunks before entering the atrium sinistrum of the heart, or aberrant veins which emptied into the Vena cava superior, the Vena cava inferior or some one of their branches.

While making a series of study corrosions of the blood vessels of the lung of the dog and their relation to the bronchial tree, Professor Miller called my attention to an anomaly within the lung which seems to be unique.

The lung of the dog consists of four lobes on the right side and of two on the left side. The lobus superior (oral) of the left side is incompletely subdivided into two lobes by a deep notch. The first of these I shall, for convenience of description, designate the apical lobe; the second, the left cardiac lobe. The first hyparterial branch given off by the left bronchus almost immediately divides (figs. 1 and 2) into two main branches which pass, the one to the apical lobe (the apical bronchus), the other to the left cardiac lobe (the left cardiac bronchus).

In the normal lung the blood is returned from the left lobus superior (fig. 1) by two main venous trunks which are situated ventral to, and slightly removed from, the corresponding branches of the bronchial tree. In the angle between the two main bronchi a small vein is seen (fig. 1) which returns the blood from a portion of the first branch given off by the apical bronchus. This vein passes behind (dorsal to) the bronchial

stem going to the aboral portion of the lobus superior, the left cardiac lobe, and joins the main venous trunk which accompanies this bronchus. It is necessary to note carefully the origin and position of this small vein, for it apparently plays an important rôle in explaining the course of the anomaly.

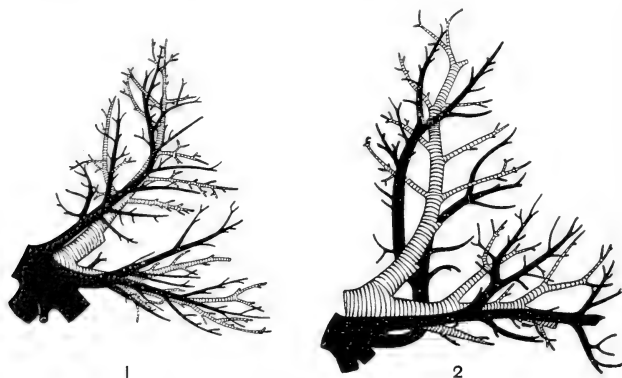


Fig. 1 Celloidin corrosion of the lobus superior of the left lung of a dog showing the normal distribution of the Venae pulmonales and their relation to the apical bronchus and the left cardiac bronchus. Note the small vein in the angle between the two bronchi. Its connection with the cardiac vein is not shown. In this figure and in figure 2 some of the bronchial branches have been omitted for the sake of clearness.

Fig. 2 Celloidin corrosion of the lobus superior of the left lung of a dog in which an anomalous distribution of the Venae pulmonales was found in the apical lobe. Note how the apical vein forms half of a spiral turn about the bronchus and eventually joins the vein coming from the aboral portion of the lobus superior.

The first portion of the anomalous vein follows closely the course of the normal vein; but as soon as the two distal radicles have united the vein swings away from the bronchus towards the facies mediastinalis (fig. 2). It then passes dorsal to the Arteria pulmonalis and the bronchus forming half of a spiral turn about these structures, and passing dorsal to the left cardiac bronchus, empties into the vein which accompanies the latter.

Only one small branch joins the lateral side of the vein throughout its unusual course. On the mesial side the branches have a normal arrangement. As the vein curves behind the artery and the apical bronchus it is joined by a branch of considerable size which is formed by venous radicles coming from the area that is usually drained by branches which join the lateral surface of the apical vein, when it takes its normal course, and by radicles which normally form the small vein found in the angle between the apical bronchus and the left cardiac bronchus. The trunk formed by the union of these two branches follows, as stated above, the course taken by the small vein found at this point in the normal distribution of the veins.

The following explanation of the anomaly seems to be the rational one. During the development of the pulmonary venous system some obstruction to, or atrophy of, the vessels which usually form the apical vein took place. Changes were thus brought about in the capillary bed and secondary trunks were formed which connected with the small vein present in the normal condition in the angle between the apical bronchus and the left cardiac bronchus; as a result this vein became the main trunk and returned all the blood from the apical bronchus.

THE ANATOMY OF A DOUBLE PIG, SYNCEPHALUS THORACOPAGUS, WITH ESPECIAL CONSIDER- ATION OF THE GENETIC SIGNIFICANCE OF THE CIRCULATORY APPARATUS

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TEN FIGURES

INTRODUCTION

The following case of syncephalus or Janiceps asymmetros, is interesting on account of first, the unusual possession of two distinct hearts each contained in a separate pericardium; second, to the subsequent fusion of the aortic arches, of both hearts, with each other, in which there were persistency or degeneration of certain integers to form the anomalous condition of the circulatory apparatus; and third, to the possession of two complete cerebro-spinal axes.

According to Fischer ('04) there were 50 human and about 80 cases in the domestic animals of the syncephalus class, recorded in teratological literature. However, I have been able to find only one case of a double pig that shows a close resemblance to the specimen here dissected.

Wymann ('61) dissected a syncephalic pig presenting the following characteristics.

Extract

"A single head in which the nervous system is made up of the right hemisphere of one brain and the left hemisphere of the other with an intermediate compound one. There is a single optic thalamus and striated body, below this the nervous system is double with a distinct cerebellum for each lateral cerebral hemisphere and two spinal cords. The head possesses two lateral ears and a posterior compound one. There are three nostrils on the snout. There are two sets of upper extremities. The body is fused from above downwards to the umbilicus; from the latter point the compound body divides into a right and left body with two distinct sets of inferior extremities. The thyroid, lungs, liver, kidneys, spleen and genitals are double. The heart is compound. The alimentary canal tract is single to the lower one-third of the ileum and from there it is double."

The chief differences as will be noted in the following text from the above description by Wymann, will be found in the nervous system and in the heart. He does not describe the remainder of the blood vascular system, so no comparison may be made there.

In cases of complete dicephalus, there are found two distinct nervous systems in their cephalic aspect at least, and generally two hearts. But in no case in the literature on syncephalus, of the Janiceps asymmetros type, to which the writer had access, either in the domestic animals or in man, was he able to find a specimen recorded possessing two distinct cerebro-spinal axes, and two distinct hearts enclosed in separate pericardia with the anomalous condition of the blood vascular system as here found.

A few cases of double monsters in other domestic animals which show some external or internal feature in common with the double pig here studied are described by Dareste ('52) in the cat, Gurlt ('32) cat, Mitchell ('90-'91) chick, McIntosh ('68) cat, Pilcher ('80) pup, Reese ('11) cat.

The following is a partial list of the cases consulted on syncephalus in man, Braun ('79), Caillé ('89-'90-'91), Debiene et Dutilleul ('90), Godson ('68-'70), Hilliard ('80), Hirst and Piersol ('93), Hue ('65-'70), Mayor ('81-'82), McLaurin ('80-'81), von Swieciecki ('87), Walter ('89).

The specimen that is the subject of this paper was given to the author in 1914 by Dr. J. S. Foote, professor of histopathology, Creighton Medical College. It was presented to the department of pathology by Drs. Sumney and Hellwig of Omaha seven years ago. From the degree of development of the external body form, the hair, extent of descent of the testicles, palpebral fissures and the foramen ovale, the monster corresponded to the appearance of a fifteen week fetus. The normal period of gestation for the pig is about one hundred and twenty days or roughly speaking seventeen weeks.

The specimen was covered entirely by white hair and weighed 500 grams. The right body is longer than the left one as can be verified by reference to the skiagraph, figure 9. The former measured 20 cm., the latter 18 cm. in the crown and rump line.

TOPOGRAPHICAL ANATOMY

Ventral aspect (fig. 1). It is readily apparent that the heads of the monster were turned laterad, the left head to the left and the right one to the right, and fused to form a single compound head with a ventral compound face and a dorsal rudimentary one. The larger ventral compound face is made up of the right side of one head and the left side of the other. The snout has four nostrils. The two well formed ones belong to the left head and the two rudimentary ones belong to the right. The left and right eyes belong to the left and right heads respectively.

The bodies are joined venter to venter. The fusion extends cephalocaudad to the umbilicus from which point caudad the bodies are distinct and normal. There is but one umbilical cord which contains four arteries, and two veins. This cord is located on a plane mesiad between the two bodies rather than on a plane passing dorso-ventrad through the ventral thoracopagic region. These vessels are distinct



Figure 1

for the remainder of the cord left intact with the monster; which was for a length of 6 cm.

External examination proved both bodies to be males; this being verified upon dissection.

Dorsal aspect (fig. 2). Upon the mesio-cephalic aspect of the fused heads is seen an oval pit devoid of hair which marks the end of the proboscis of a cyclops; this proboscis was formed by the fusion of the

right eye of the left head and the left eye of the right one. At the base of the skull a pair of small ears of the dorsal face are found close together, which bear the same relationships to the compound skulls as do the eyes. The two dorsal fore limbs are separate integers, the left being the right fore limb of the left body and the right the left fore limb of the right body. The two separate bodies below the bifurcation of the monster are normal.



Figure 2

INTERNAL ANATOMY

Although the muscles were dissected and studied, no attempt will be made to record here the detailed findings.

Respiratory, digestive and genito-urinary systems (fig. 3). There was a ventral and a dorsal pair of lungs. The former possessed a three lobe right and a two lobe left lung; the latter, a three lobed left and a

two lobed right lung. Each pair of pulmones is composed of integers heterogeneous in origin. The ventral pair comprises the right and left lung of the right and left bodies respectively; the dorsal, of the left and right lung respectively of the right and left bodies. Each pair of lungs possesses a corresponding trachea which leads cephalad into its respective larynx. Each of the latter was surmounted by an epiglottis. The larynges were at the same level on a mid-dorso-ventral line.

The tongue was compound. At the location of the foramen cecum of the right integer there was a pedicle 1 cm. long; on the distal end there was located a globular structure. Upon section this proved to be thyroid glandular tissue. The left thyroid gland was present but the right was absent from its normal locations in the neck. Cephalad the opening into the oesophagus was located between the two larynges. The alimentary tract was single to within 16 cm. of the cecum. At the former point the ileum bifurcated, each integer leading to its respective ileo-cecal valve. However, this single tract possessed a much larger ileum than is normally found even in a post-partum pig of two or three weeks of age. Each portion of the now double alimentary tract was normal for its corresponding body.

There were two livers the right being larger than the left. These were located considerably more caudad than normally. The common bile-duct from each gall-bladder was joined by the pancreatic duct of the same side 0.25 cm. before the entrance into the duodenal wall. There was a normal pancreas for each body.

The genito-urinary system was double each entity being normal. The testicles were undescended all four being located respectively in an inguinal canal just internal to the external abdominal ring.

CIRCULATORY SYSTEM

Ventral aspect (schematic (fig. 4)). There was one large heart situated ventrad within the thorax in a separate pericardium. Dorsad and to the left was found a heart one-quarter of the size of the ventral one. The former evidently had been crowded into the position it occupied in the right portion of the thoracic cavity of the left body by the much larger and more vigorous ventral cor. The smaller one also possessed a separate pericardium. The auricular cavities of each heart were normal in structure. However in both, the auricles were connected by an abnormally enlarged foramen ovale due to the failure of complete development of the septa prima and secunda. The ventricular cavities of both inter-communicated due to the lack of development of the interventricular septa.

The large ascending aorta of the ventral heart bifurcated 1.5 cm. from its origin into a larger right aortic arch and a smaller left one. At the junction of the left aortic arch with its corresponding ductus arteriosus, the left and right subclavian arteries of the left body are

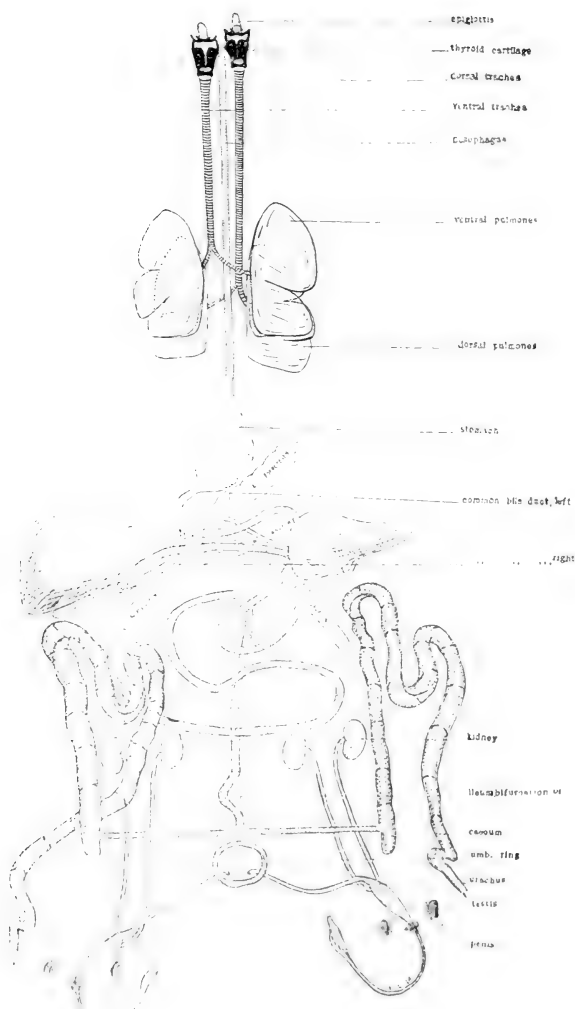


Figure 3

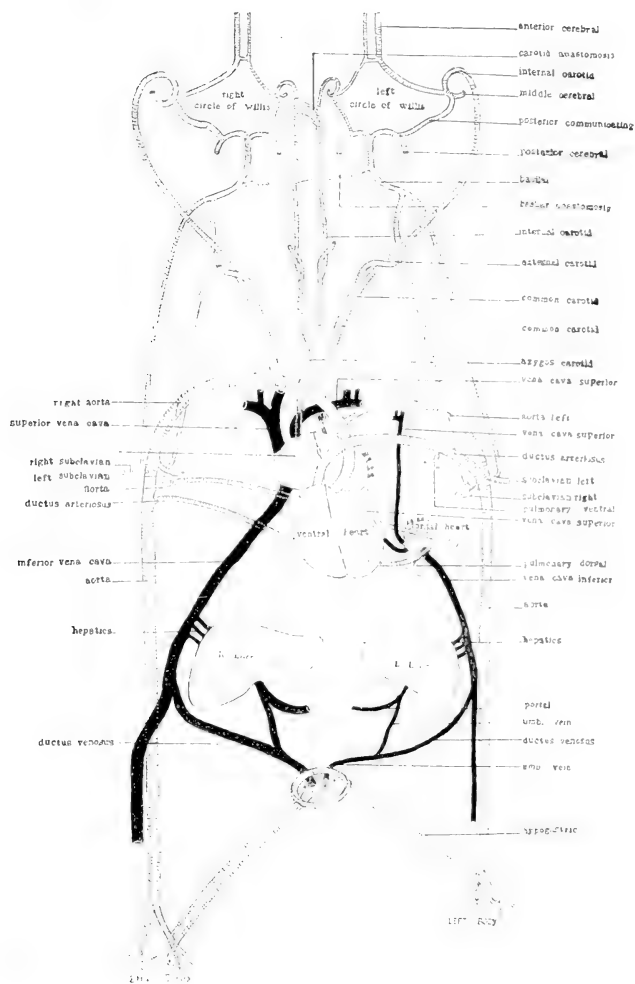


Figure 4

given off. Coming from the latter arteries their respective vertebral branches are found.

The right aortic arch of the ventral heart, just proximal to its junction with the left aortic arch of the dorsal heart, gives off the right and left subelavian arteries of the right body. From the latter vessels their corresponding vertebrals are derived. One cm. caudad to the junction referred to above, the ductus arteriosus of the dorsal heart joins the descending aorta of the right body. The latter vessel, as will be seen later, belongs genetically to the dorsal heart.

From the left aortic arch of the dorsal heart a single vessel arises which is called here the azygos carotid. Four cm. from its origin it bifurcates into the two common carotids. The internal carotids of these latter vessels, go to form the mesial aspects of their respective circles of Willis. The left aspect of the left circle of Willis and the right aspect of the right circle of Willis derive their vascular supply from the internal carotids which are branches of the common carotids of the right aortic arch of the ventral heart.

Four hypogastric arteries leave the umbilical ring, one from each of the four internal iliac arteries. There are two umbilical veins which are distributed normally one to each body.

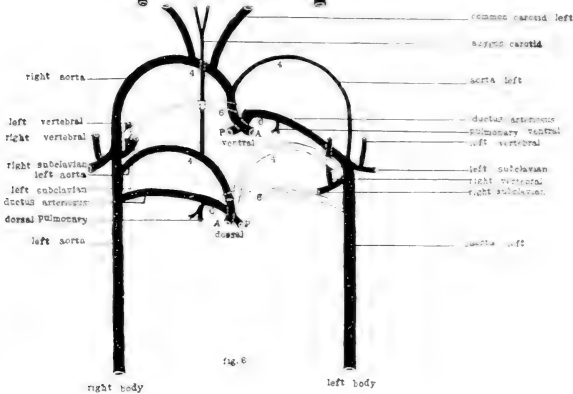
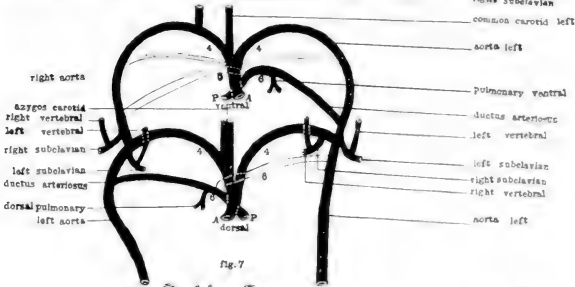
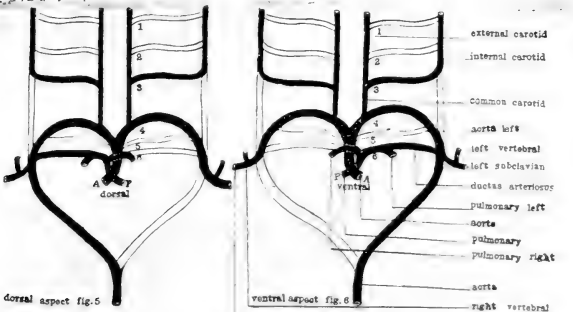
The intercostal and lumbar arteries although not depicted in figure 4 are distributed in a normal manner from each descending aorta.

There are two spleens.

Genetic significance of the circulatory apparatus (figs. 5, 6, 7, 8). In order to simplify the understanding of the means by which such an anomalous condition of the circulation was formed, it will be necessary, in the first place, to consider the normal aortic arches of two hearts as presented in figures 5 and 6. The former depicts the normal arches of a heart from the dorsal aspect; the latter those from the ventral view. In the fusion of two embryonic areas or the splitting of a single one to form the monster, the bodies were ultimately brought venter to venter and conjugated; as a consequence the ventral surfaces of each set of arches were brought into apposition.

After the fusion and the resultant abnormal alteration of origin of some of the large vessels, we then would have the condition of the circulation as represented in figure 7. Here we find the right and left aortic arches of each heart still persisting. Two common carotids come off abnormally from the right aortic arch of the ventral heart and a single trunk called, as previously stated, the azygos carotid, from the left aortic arch of the dorsal one. It is important to remember that genetically the right common carotid of the right aortic arch of the ventral belongs to the dorsal heart; and that the smaller left common carotid of the azygos carotid of the dorsal heart belongs to the ventral one. As schematically shown, the subelavian arteries are still branches of their respective right and left arches.

In figure 8, we find the complete degeneration of the proximal portion of the right aortic arch of the dorsal heart; the subelavian artery of this vessel is now a trunk of the descending aorta of the ventral



heart and supplies the right extremity of the left body. The left subclavian of the left aortic arch of the dorsal heart is abnormally a branch of the first part of the descending aorta of the ventral heart and furnishes the blood supply to the left extremity of the right body.

From the above embryological consideration it is seen that the larger ventral heart belongs genetically to the left body whereas the smaller dorsal heart belongs to the right body. However, the dorsal heart has been rotated and crowded from its earlier position into the right side of the thoracic cavity of the left body by the larger and more vigorous ventral heart. The ventral surface of the dorsal heart is now directed ventrad, in reference to the double monster, instead of dorsad. As a consequence the relationship of its great vessels is considerably altered.

The right and left aortic arches of the ventral heart have persisted, the right being considerably the larger of the two as previously pointed out. The right by joining the descending aorta of the dorsal heart form a good size trunk, slightly larger than the left one. This is due to the fact that both hearts contribute to the descending aorta of the right body and only the smaller left aortic arch and its corresponding ductus arteriosus of the ventral heart go to form the descending aorta of the left body. It is therefore clearly seen that the right and larger body receives the greater blood supply, "due to the influences which have brought about a more abundant growth of capillaries," Evans ('12).

Developmentally the vessels of the dorsal heart supply the right side of the left body and the left side of the right body whereas those of the ventral one, supply the left side of the left body and the right side of the right body. In other words, the larger ventral heart supplies the ventral aspect of the syncephalo-thoracopagic regions, of the double monster and the smaller dorsal heart genetically contributes the vessels distributed to the dorsal aspect. In the lumbar, pelvic and caudal extremities of each body, the vessels are normally distributed.

The same conditions that governed the relationship of the areas supplied by the arteries of the ventral and dorsal heart ruled over the veins draining those regions. However, the entrance of the veins can be traced to their respective hearts, whereas on the other hand, the genetic relationship at first is somewhat obscure and blotted out in regard to the arteries. Each heart possesses two superior venae cavae representing the persistent anterior cardinal veins which emptied separately at the right and left lateral poles of the sinus venosus.

Although figures 5 and 6 are schematically represented as normal arches, it is by no means the intention of the writer to convey the impression that two absolutely normal aortic arches, developed to the stage as here represented, were necessarily at one time existent, or that fusion took place at this late period in development. The establishment of this anomalous circulatory apparatus was no doubt forecast in the fused capillary net-work which was the anlage of the abnormal

condition of the arches as shown in figure 8. The early indifferent endothelial vascular network of each heart fused and then the elaboration of the main trunks as depicted in figure 8 represent a physiological adaptation of this fused network to the demands of the circulation in the monster. The fortuitous location of certain channels of the net with regard to the aorta of either heart determined the enlargement and use of these paths to form the normal and abnormal vessels. It was merely for the sake of simplicity to emphasize the venter to venter apposition and fusion of the anlage of each set of aortic arches, that two developed normal sets are used in diagrams, instead of the complex antecedent network of each. As a consequence correct sequence of development of the vessels was sacrificed for simplicity.

THE SKELETON

The posterior extremities, pelvis and lumbar vertebrae were normal for each body. In the dorsal region of the left vertebral column, from the third to the ninth dorsal vertebrae, kyphosis is presented. This is undoubtedly the cause for the shorter length of the left body.

The thorax was compound, the sternum and ventral ends of the ribs of each side were so reflected and fused with their corresponding parts of the other as to form one large thoracic cavity bounded by fifty-six ribs; fourteen on the lateral aspect of each body. Each sternum, dorsal and ventral, has a double origin as is apparent from the reflection considered above. Only the upper seven ribs were attached to either sternum. The remaining seven were absolutely free, having neither cartilaginous nor bony connections at their distal ends. The thoracic appendages were normal in structure. However, the ventral pair were better developed than the dorsal pair.

The spinous processes of the cervical vertebrae were directed laterad instead of dorsad which fact may readily be verified by a reference to the skiagraph, figure 9.

The skull is compound. This is apparent for each vertebral column has its separate occipital attachment. In the fusion the interior of the compound cranium was left double. The right temporal, parietal, frontal, nasal, parietal maxillary and lateral aspect of the occipital bones of the left skull fused with the corresponding parts of the right one, to form an attenuated partition between the left and the right encephala. This line of fusion may be seen from the exterior extending through the oval depression marking the point of emission of the proboscis of the cyclops, marked 2, figure 10. The two opposed orbits were fused to form the condition of synophthalmia. The partition did not leave the normal amount of space for the development of each brain; this is apparent by the fact that the mesial hemisphere of each encephalon is smaller than the lateral one. The adjacent walls of the mesial hemispheres present parallel surfaces instead of being normally convex.



Figure 9

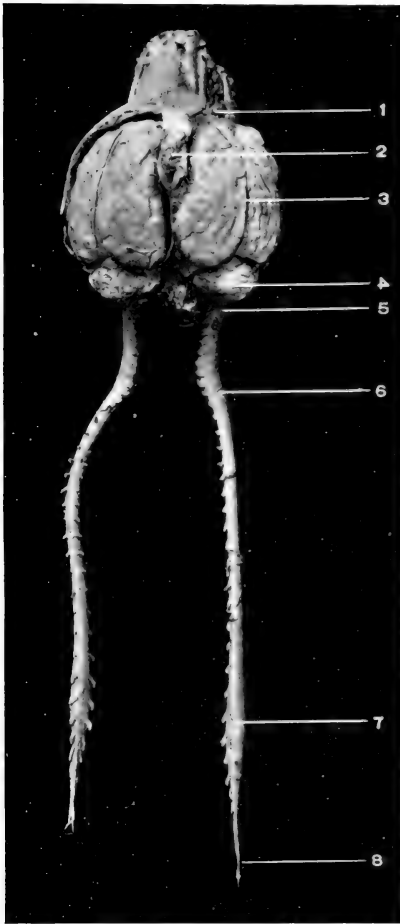


Fig. 10 Cerebro-spinal axes. Dorsal aspect. 1, olfactory bulb; 2, proboscis of the cyclops; 3, right cerebrum; 4, cerebellum; 5, medulla oblongata; 6, cervical swelling; 7, lumbar swelling; 8, cauda aquina.

CEREBRO-SPINAL AXES

There are two encephala in the double compound cavity. Each brain and stem is distinct, there being no fusion. However, the right optic nerve of the left encephalon and the left one of the right encephalon do fuse to form a compound trunk. This terminated in a small amorphous structure which represents the fused eyes of the cyclops, marked 2, figure 10, as referred to above. The optic nerve of the right lateral normal eye belongs to the right encephalon; that for the left lateral normal eye is derived from the left encephalon. Neither is seen in figure 10, due to the fact that the cerebrospinal axes were so placed that the center of focus would fall on the synophthamia. Each encephalon as a result obscured its corresponding lateral eye from view. The remainder of the cranial nerves were distinct and separate. Except for the optic fusion as here considered, there is no other connection between the cerebro-spinal axes.

The effect of kyphosis of the dorsal vertebrae on the contained cord is readily apparent in figure 10. The marked loop to the left in the left spinal cord in the upper dorsal region is clearly seen. In actual measurement both cords have the same length. The shortening is due to the loop caused by the kyphosis circle of Willis.

Each heart contributes blood to each encephalon as was seen above. The dorsal heart furnishes the blood supply to the mesial aspects of each circle of Willis, whereas, the ventral heart furnishes the blood to the lateral aspect of each circle of Willis.

In conclusion, I wish to express my sincere thanks to Dr. Foote for the specimen here recorded and to Dr. Tyler, professor of Roentgenology of Creighton Medical College, for the skiagraph of the skeleton, figure 9.

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A DEVICE TO INCREASE THE EFFICIENCY AND EASE OF MANIPULATION OF THE FINE ADJUST- MENT OF THE MICROSCOPE

ROBERT T. HANCE

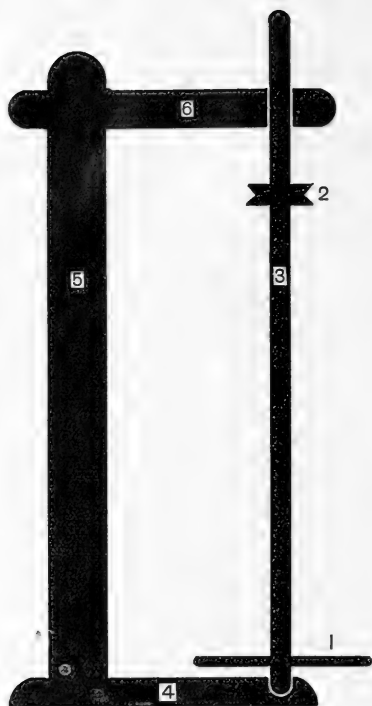
Zoological Laboratory, University of Pennsylvania

ONE FIGURE

The use of high powered microscopic lenses requires the hand to be constantly on the fine adjustment. When the study is continued for several hours at a time the hand and arm doing the focusing, even though the latter is supported at the elbow on the table or chair arm, become extremely fatigued. To obviate this strain the apparatus figured below was devised. Although the role it was originally intended to fill was merely to facilitate the use of the fine adjustment, it has, since its installment, not only been of aid in this way but has greatly diminished several annoying features that are frequently connected with high power work. For this reason it seemed worth while to bring the attachment to the attention of microscopists.

The device as figured is for use with microscopes which have the more common 'top' fine adjustment although it would be a simple matter to construct a similar apparatus for use with the side wheel type. No dimensions are given, as these must be controlled by the height of the particular microscope the attachment is intended to be used with. The illustration, which is a side view, is just one-half the size of the one I have in use. The entire apparatus is made of wood and it is simpler to use dowel sticks of various diameters for the pillar (5), the arm (6), and the shaft (3), although other material could be used satisfactorily. The focusing wheel (1) is a disc cut from lumber about one-eighth of an inch thick. It is of advantage to have this wheel large (two to two and one-half inches) and thin, so that it may be easily gripped and turned with either the thumb or fingers. The edge of this disc is rounded and notches are filed into it at intervals of about one-quarter of an inch to prevent the fingers gripping it from slipping. The grooved wheel (2) is made by gluing together two discs about one inch in diameter (cut from thin lumber) whose adjacent rims have been beveled. It is well to have the channel or groove rather deep so as to prevent the belt from jumping when the microscope is inclined.

Care, of course, must be taken to center the hole in the arm (6) through which the shaft (3) passes and the socket in the base (4) into which the end of the shaft slips. Smoothness of action is obtained by soaking that part of the shaft that is in contact with the arm, and the hole in the arm through which the shaft passes, with paraffin. The socket in the base is filled with paraffin so that the shaft is resting in a



tightly packed bed of this material. I am indebted to Dr. C. E. McClung for suggesting this method of lubrication, which results in a very soft, smooth movement.

In use the apparatus is screwed to the table (in my case to the drawing board on which the microscope rests) with the pillar (5) away from the observer. A belt composed of string which will not stretch (such as braided fish line) is passed around the pulley (2) and the focusing wheel of the microscope. The microscope may be used in any position. The focusing arm lies flat on the arm of the chair or the table and the focusing wheel (1) is within easy access.

Since the pulley (2) is made smaller than the fine adjustment wheel of the microscope the sensitiveness of focus is increased. The delicate focusing of high powers has been found to be much steadier with this attachment. The annoying feature of the field under observation being a trifle displaced while drawing, due to the hand on the focusing wheel jarring the microscope (however little), is largely eliminated with the use of the device described as it is now unnecessary to directly touch any part of the microscope.

ON THE MECHANISM OF MORPHOLOGICAL DIFFERENTIATION IN THE NERVOUS SYSTEM

II. THE RELATION BETWEEN COMPRESSION AND THE DEVELOPMENT OF A SERIES OF VESICLES.

OTTO C. GLASER

From the Zoological Laboratory of the University of Michigan

EIGHT TEXT FIGURES AND THREE PLATES

I. INTRODUCTION

The simple neural tube mentioned so frequently in embryological literature is really no more than a convenient fiction. Practically it cannot be found at any stage of development. The mere differences in the amounts of tissue in the anterior and posterior ends of the neural plate alone preclude this possibility, but in addition, long before the separation of the plate from its extra-neural ectoderm is complete, the developing 'tube' prefigures a serial differentiation that culminates in a succession of vesicles, within limits, highly constant for the vertebrate nervous system, and, as one of its basic attributes, calling for explanation.

The stages in embryogenesis upon which a study of this problem must be based, are necessarily as commonplace as those dealt with in my first paper¹ yet despite their familiarity with this period of development, embryologists do not tell us why the nervous system differentiates a series of vesicles, and particularly why there are five such fundamental divisions in its anterior end. Many do not even ask the necessary questions although His, over forty years ago, considered the subject as legitimately within the province of the student of development.

¹ On the Mechanism of Morphological Differentiation in the Nervous System.

1. The Transformation of a Neural Plate into a Neural Tube. *Anat. Rec.*, vol. 8, pp. 525-551, 1914.

In fact His² himself attempted to explain serial differentiation. The analysis, in *Unsere Körperform*, begins with the 'closed neural tube,' whose anterior end, as indicated in figure 1, is larger than its posterior, and is characterized by three enlargements—the precursors of the five secondary brain vesicles.

In a later stage, figure 2, His illustrates the relative positions of the five secondary vesicles in the flexed state. The curve, typical of cranial flexure, is divided into a 'Brückenkrümmung,' involving the fifth and fourth vesicles; a 'Mittelwölbung,' involving the mid brain; and the 'Hackenkrümmung,' involving the second and first. At the extreme anterior point of the first vesicle is the 'Trichterfortsatz' Tr.

His then attempts to show how this form can be derived from that given in figure 1. The brain, according to the argument, begins as a tube whose comparatively large lumen is enclosed by moderately elastic walls. These conditions are identical with those given in a piece of rubber tubing.

If the edge of a rubber tube is made fast by threads in the manner indicated in figure 3 longitudinal compression will pro-

Fig. 1 After His. Chick embryo of the second day. *H*, brain, with three vesicles indicated; *Ag*, optic vesicle; *W*, Wolffian ridge; *Uw*, somites; *Ump*, unsegmented mesodermal bands; *Am1*, head fold of the Amnion; *Am2*, lateral folds of the Amnion; *Mp*, open portion of the neural tube. The heart is indicated by means of dotted lines. Enlarged 20 ×.

Fig. 2 After His. Brain of chick embryo of the third day. *Ag*, optic vesicle; *Vh*, forebrain; *Tr*, 'Trichterfortsatz'; *Zh*, interbrain; *Mh*, midbrain; *Hh*, hindbrain; *R*, location of fourth ventricle; *Br*, 'Brückenkrümmung'; *Nh*, after-brain; *Gh*, auditory vesicle. Enlarged 30 ×.

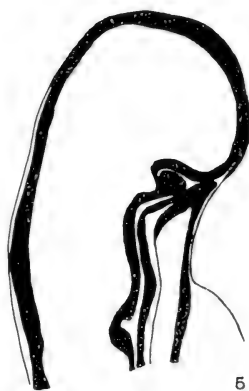
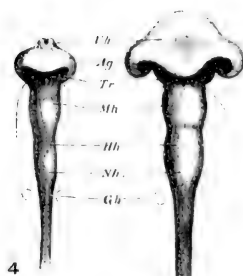
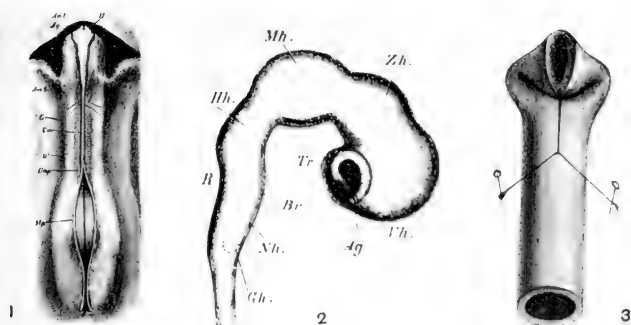
Fig. 3 After His. Rubber tube whose upper end has been drawn backwards by means of a thread.

Fig. 4 After His. For explanation of legends see figure 2. The dotted line indicates the anterior limits of the foregut.

Fig. 5 Sagittal section through chick-head about 38 hours old, showing the relations of foregut, floor of the second brain vesicle, and the anterior end of the notochord.

Fig. 6 After His. Upper figure, head of embryonic pike; middle, of trout, and lower, dorsal view of same. Legends as in figure 2. *Rg*, olfactory pit.

² Wilhelm His: *Unsere Körperform und das Physiologische Problem ihrer Entstehung*. F. C. W. Vogel, Leipzig, 1874. Achter Brief, pp. 93-104.



duce an abrupt bend just behind the level of fixation, and there will appear in this region a pair of lateral swellings, whereas on one side—either the upper or the lower, as the case may be,—a sharp groove, deepest in the median line, shallower toward the edges of the sidewise expansions, will cross the tube in a transverse curve with concave face forward.

His points out how exactly this form duplicates the ventral surface of the second vesicle in the stage of development depicted in figure 1.

The suggestion that identity of form in the two sets of cases is the result of identity in the mechanical conditions under which they were produced is very much strengthened, as His emphasized, by the union "welche durch den Axenstrang und später durch die aus ihm entstandene chorda dorsalis zwischen der Medullarplatte und dem Darmdrüsenblatte, längs der Mittellinie unterhalten wird."²

This union later undergoes the following modifications: first the entoderm separates from the chord,

und, viel später, diese vom Medullarrohre Am innigsten ist die Verbindung durch zwischengelagerte Masse zwischen dem Ursprünglich vordersten Rande der Medullarplatte und vom vorderen Ende des Vorderdarmes. Die Verbindung ist hier eine so innige, dass, wenn in sehr später Zeit der Vorderdarm vom Gehirn sich trennt, die Trennung nicht im Verbindungsstücke geschieht, sondern in der Continuität des Vorderdarmes selbst. Ein kleines Stück von diesem bleibt als Vorderer Lappen der Hypophysis in dauernder Verbindung mit dem Gehirn.⁴

His continues,

Es wächst aber das Medullarrohr, und speciell das Gehirn rascher in die Länge als der Vorderdarm; da es nicht zu einer Trennung beider Theile kommt, so muss der längere Theil sich Krümmen, und müssen ferner die unmittelbaren Folgen der Zerrung in den mit einander verbundenen Strecken des Vorderdarms sowohl, als des Medullarrohres zu Tage treten. Beides trifft in sehr prägnanter Weise ein, nicht allein erhebt sich das Medullarrohr über dem Vorderdarm in wachsendem Bogen, sondern es ziehen sich an beiden Theilen die verbundenen Enden trichterförmig aus, wir bekommen auf die Weise am

² Loc. cit., p. 99.

⁴ Loc. cit., p. 100.

Gehirn den oben betrachteten Trichterfortsatz (fig. 2), am Vordarm die bekannte sog. Rathke'sche Tasche.⁵

The union of the 'trichterfortsatz' with Rathke's pouch is, under the conditions of growth, mechanically the exact equivalent of the piece of string in figure 3. The striking similarity of the forms produced in the two cases scarcely requires comment and may almost be considered proof of the correctness of the assumptions. One factor, not emphasized by His, however, is the notochord. Given the fusion between 'Trichterfortsatz' and Rathke's pouch, flexure resulting from the faster growth of the nervous system would be accentuated by the notochord whose anterior end would play the rôle of a fulcrum about which the curvature would take place.

But His' account is not free from anachronisms, nor will it bear a too close scrutiny from the standpoint of comparative anatomy and embryology. The final conclusion which he wishes drawn, "von der Abhängigkeit in welcher die Gehirngliederung von den auftretenden Longitudinalbiegungen des Organs steht,"⁶ does not follow. If this were correct, sharpness in the demarcation of the vesicles from one another should vary directly with the degree of cranial flexure in individual as well as comparative ontogeny. This, for the first case is not strictly true, and, for the second, scarcely at all, since the vesicles are distinct before cranial flexure, and forms exhibiting a high degree of flexure have their vesicles no more sharply delimited than those in which the bending of the embryonic head is never very marked. I have no reason to doubt that His was correct with respect to the origin of both the flexure, and the lateral expansions, or optic lobes, but the differentiation of the vesicles themselves cannot be explained as the result of flexure.

II. ON THE EFFECTS OF DIFFERENTIAL GROWTH

It is clear that His considered differential growth the cause of flexure, and flexure the cause of vesiculation. Since, however, only the first vesicle with its ventral groove and lateral expan-

⁵ Loc. cit., p. 100.

⁶ Loc. cit., p. 104.

sions can be accounted for by flexure, the case for this factor is hardly made out.

From his manipulation of rubber models, forced in various ways to simulate special regions of the nervous system, as well as from the general tenor of the argument, it is likely that His would not have denied that differential growth, in the early stages of flexure, results in some sort of compression in the longitudinal axis. Be this as it may, it is certain that he attributed to this factor not only flexure, but also the changes in relative position which the vesicles undergo during later stages of development. Concerning the head of the embryonic pike, figure 6, he writes: "Durch die wachsende Zusammenschiebung der Theile ist der hintere Hirnabschnitt, oder das Nachhirn unter die davor liegende Anlage des Kleinhirns, und diese unter diejenige des Mittelhirns geschoben worden."⁷

III. HIS' DEMONSTRATION OF DIFFERENTIAL GROWTH

His attempted to demonstrate the differential growth of the nervous system on chick embryos ranging roughly from the twenty-fourth to the ninety-sixth hour of incubation.⁸ The method consisted in comparing at four levels, those of the eye, the 'Gehirnblase,' and the first pair of somites, the areas in transverse section of the 'neural tube'—thought of as spread out flat, and the ectoderm, measured from the median axis to the point of fusion with the lateral muscle plate. On this basis, he was able to convince himself that the nervous system actually grows at a faster rate than the tissues with which it was compared.

Although this method may be capable of demonstrating the point, it is certainly not able to show that differential growth in width is accompanied by differential growth in length. The

⁷ Loc. cit., p. 103.

⁸ According to his own statements, the youngest stage used was of the second day, but his figure with its nine pairs of somites hardly bears this out. See figure 1 of the present paper.

association of the two is of course very probable, but by no means necessary.

Differential growth in length can be determined directly only by measurements in the long axis, and if the values so secured have a certain sense and magnitude, they may be made the basis for inferences concerning compression in that axis.

IV. METHOD OF DETERMINING COMPRESSION IN THE LONG AXIS

In order to discover the presence or absence of compression in the long axis it is necessary to find at least one measurable relation which differential growth either changes or brings about. This relation must be longitudinally effective, widely applicable, in magnitude independent of absolute measurements, and finally, capable of exact expression.

Many attempts were made to satisfy these conditions before it was found that the necessary data, accuracy, and reliability were obtainable in a very simple way.

With the camera lucida, optical sections of embryonic chick heads, at convenient magnification and a focus giving maximal outlines, were carefully traced. About these outlines I then erected the system of lines indicated in figure 7.

The base of this system is a line tangential to the anterior face of the first pair of somites. Upon this base perpendiculars, themselves tangential to the sides of the head at its widest level, were erected, and finally, parallel with the somitic base line, a tangent to the anterior edge of the head. In this way the entire cephalic region is included within the area of a rectangle.

The purpose of these preliminaries is to get a measure of the length of the head. Without the rectangle it would be easy enough to find an anterior point of reference, but it is never possible to tell exactly where the posterior limit of the head is. In fact in the early stages this grades so insensibly into the body that any posterior limit permitting comparison between various embryos and different stages, of necessity has to be chosen arbitrarily. If then an arbitrary point is imposed by the conditions of the case, it is best to choose one whose relations to the rest

of the embryo are not only significant, but also likely to present the greatest relative constancy. The somites possess these qualifications more than any other structures, and so I took the longer sides of the cephalic rectangle which rests upon the first pair, not as *the*, but as *a* measure of head length.⁹ Since we desire a measure of differences in the rate of linear growth be-



Fig. 7 Diagram to illustrate the cephalic rectangle. The line tangential to the anterior face of the first pair of somites, which are indicated in solid black, is the somitic base line. The perpendiculars erected upon this line are tangential to the head at its widest points. The head-length was arbitrarily determined along these perpendiculars as the distance between the somitic base line and the parallel tangent to the anterior surface of the head.

⁹ To carry out these and the subsequent measurements on live embryos presents, for the present, insuperable difficulties. Fixed material, no doubt, differs in absolute values from the living, and it is possible that the relations between measurements which I shall discuss, are also not the same. However, absolute values need not concern us at all in the present connection, and if the relative values upon which my argument rests were seriously changed, I should hardly expect the constancy in sense which they exhibit.

tween the neural mass and the head, the simplest method is to compare the length of the head with the length of the nervous system, in various stages of development, measured from the somitic tangent as a base. Provided the head of the embryo exhibits no serious irregularities, its length may be determined arbitrarily in the manner indicated above, but, on account of the vesicles, the length of the 'brain' cannot be given by the cephalic rectangle.

Several methods involving the volume of the brain were tried but discarded in favor of one which in addition to simplicity, is capable of giving exactly and directly the very information that is wanted. All that is necessary is to determine by means of a map-measurer the perimeter of the nervous system beginning at its intersection with the somitic tangent on one side and ending at the corresponding point on the other. The length of the nervous system will be half this perimeter.¹⁰

V. THE NEURO-CEPHALIC QUOTIENT

With the aid of these measurements it is possible to express in the form of a fraction the relation in each stage between the length of the head and that of the nervous system. The fraction chosen is derived by dividing half the neural perimeter into the head length and tells us how many units of head length, within the limits of the cephalic rectangle, are available for every unit of length in the nervous system. This fraction I shall call the Neuro-Cephalic Quotient.

Very few embryos approach the ideals of rectitude and symmetry depicted in text-books and wall-charts. Out of the entire collection with which I have worked I have been able to find only eight justly to be characterized as diagrammatic. The outlines of these are reproduced in plate 1, figures A, B, C, D, E, F, G, and H, whereas the number of somites, and the corresponding neuro-cephalic quotients are given in table 1.

¹⁰ Since this method is applicable to the nervous system it might be asked why I did not apply it also to the head instead of relying on the long sides of the cephalic rectangle. The answer is, because the perimeter of the head in early stages is distinct only in the anterior region.

On account of the small number of cases the scientific value of this particular table is negligible. Nevertheless it suggests certain problems and questions which must be dealt with before we can arrive at a just estimate of the significance of the quotient.

The values, taken for the time being as they stand, seem to indicate that when diagrammatic embryos are arranged in series according to somites—or, in other words, according to age and degree of development, the size of the quotient will be found to vary inversely with the number of somitic units. This, if correct, means that as development goes on the amount of head length into which a given length of nervous system must fit, decreases progressively.

TABLE I

EMBRYO	NUMBER OF SOMITES	QUOTIENT
A.....	2	1.485
B.....	8	0.902
C.....	9	0.877
D.....	9	0.837
E.....	10	0.820
F.....	10	0.820
G.....	10	0.846
H.....	11	0.772

Given this condition, differentiations of some sort, spreading, collapse, telescoping, or vesiculation, are to be expected. Leaving aside, for the moment, the question why vesiculation prevails, let me explain why I choose to consider first these rare diagrammatic forms. The reason is very simple: because in these, the presence of a high degree of symmetry indicates that the developmental processes in general have been in nearly perfect balance, and have given a result relatively or quite free from complications calling for special explanations. Briefly, these forms are the simplest available.

Granted the advantage of simplicity—we must ask why a well balanced development gives rise to the obvious discrepancies which the table exhibits and furthermore why it is itself so rare.

The effect of error introduced by the various technical methods employed in preparing the embryos for study cannot be denied,

nor can I claim for my measurements a maximal degree of accuracy. Nevertheless I see no reason for suspecting greater errors than inhere in embryological and biometric work in general. For reasons which I shall attempt to make clear, I believe that the major discrepancies inhere in the material itself.

Referring to the table, the general sense of the values is very obvious. There is, however, no absolute proportionality between somitic increase and the shrinking quotient. Furthermore, embryos like C and D, each with nine somites, have quotients separated by a wider margin than C and G. A series arranged according to somites, even in diagrammatic forms, does not coincide absolutely with a series based on quotients. That there is a general coincidence, however, will hardly be denied.

It was the desire to find embryos in which just this sort of discrepancy could be expected to assert itself in minimal degree that lead me to select the most diagrammatic forms for separate treatment. But even in these complete elimination of discords is impossible. If now the lack of harmony between theory and practice is to be sought in the embryo itself rather than in the methods by which it was handled, we must analyze our material in an attempt to get at the real explanation.

Such explanation will be found, I think, when we realize the true nature of the developmental process itself. To be alive is to solve a constellation of interlocking problems in equilibration. In the adult, departures from balance occur within comparatively restricted compass, and, being for the most part quickly reversed, result in few or relatively unimportant morphogenetic changes;¹¹ in the embryo, on the contrary, the excursions are often very wide. Indeed it is hardly too much to say that a developing organism 'blunders' from one crisis to another, until gradually, by the narrowing of its 'horizon,' it reaches that state of relative stability which is characteristic of the adult. Nothing that happens in the fully developed organism can be compared with the multiplicity and complexity of the immediate and remote adjustments consequent upon the differentiation of

¹¹ See Glaser, *The Basis of Individuality in Organisms*. Science, vol. 44, pp. 219-224.

the germ layers. From the dynamic standpoint, development might be defined as the symptom of an organic instability in which departures from exact balance occur within limits so wide as to escape fatality by only a narrow margin.

This granted, it follows that the sum of opportunities within the developing system is exceedingly great. Although unquestionably exact and theoretically predictable in all its details, embryogenesis, within the boundaries of what is 'normal,' nevertheless, varies tremendously. It need occasion no surprise then if we find differences in the several tissue-maneuvres, or in the exact time of onset of this, that, or the other process. Of several embryos which might be expected to exhibit identical conditions in all respects, one may lead or lag in the morphogenetics of its nervous system, a second in that of its somites, and another in its circulatory equipment. In fact the early discrepancies or temporary misfits of development may at any instant simulate disorganization. By this it is not intended to suggest that 'normal' embryogenesis is strictly a matter of chance, but only that its 'administration' appears relatively loose. To us this looseness is emphasized subjectively because we remain ignorant of so large a share of the elements underlying the process. We may be sure, however, that the minor errors of development sooner or later receive adequate correction for the end results of embryogenesis are precise and give an organism which actually describes the genetic constitution of its ancestors.¹²

Taking development as it is, our quotient, to have significance, must be applicable to a wide range of cases, which, no matter how they may deviate from the diagrammatic, nevertheless cannot be considered otherwise than normal. Within this range, if our preliminary test is to be trusted, we should find the same general relations exhibited by the ideal embryos. However, since the measurements upon which the quotient rests, themselves bear no obviously immediate relation to the factors upon which the production of somites depends, we should not expect absolute correspondence between somitic increase and a falling

¹² See Note 11.

quotient. All that we are entitled to expect is that the quotient in general will vary inversely with the number of somites.

If the quotient is a true indicator of compression in the longitudinal axis, and if compression is related causally to serial differentiation, we are also entitled to expect some relation between this differentiation and the quotient. Here again, we should not set our minds on absolute correspondence, for quite apart from the difficulty of exact determinations in this connection, the amount of differentiation which a given degree of compression calls forth depends on many things. Age is one; what differentiations had taken place before a particular degree of compression was reached, is a second; the thickness and mechanical properties of the nervous system are a third; the rate at which the differentiations are produced is a fourth; the exact axis of maximal compression is a fifth; and no doubt there are many others. There is, however, no accurate way, particularly in the more complicated cases, of expressing the degree of differentiation. Furthermore the attempt to do so would necessitate also the consideration of that portion of the system which lies posterior to the somitic base line of the cephalic rectangle. Nevertheless, little serial differentiation should be associated with a large, and the reverse with a small quotient.

Correspondences between quotient and somites on the one hand, and quotients and differentiation on the other, do not complete the list of what we may expect. A third matter—that of aberrancies—must receive consideration.

From our present standpoint, the aberrancies theoretically to be considered are those in which the nervous system is either underdifferentiated or has erred or has been forced to err seriously in the opposite direction. In the first case we should expect relatively high, in the second, relatively low, quotients. As a matter of fact, I have not found enough cases of undifferentiation to feel warranted in giving them special prominence, but overdifferentiation is common. In fact, in one of its varieties, it is too common to leave much doubt that the resulting forms must be classified as normal. This type, characterized by the partial telescoping of the first and second vesicles exceeds in

frequency the known percentage of faulty hatchings or total failures by so large a margin as to suggest very strikingly indeed the likelihood that many nervous systems during some stage of their development exhibit temporarily, at least, too much differentiation. Cases in point are illustrated on plate 2, embryos I, J, K, L, M, N, O.

To what extent now, our several expectations are fulfilled can be seen by comparing the figures of the forms illustrated in plates 1 and 2, with the corresponding values given in table 2 in which the embryos are arranged in a series beginning with the highest and ending with the lowest quotient.

The omission from this table of 12 and 14 somite embryos is due to the fact that only one of each kind was available. The only other grounds of elimination were asymmetry or a degree of aberrancy which could have but one interpretation. Such embryos with somites and quotients indicated are referred to in table 3 and illustrated in plate 3.

If we accept table 2 as indicative of the norm for the various stages dealt with, the quotients in table 3 become significant. As can be seen by reference to plate 3, the embryos now under consideration fall into two groups—in one of these, including U, W, and X, the embryo is asymmetrical, in the other, T, V, Y, and Z, the embryos are overdifferentiated. With respect to Z, nothing definite can be said, since table 2 does not contain the quotient normal for the 27 somite stage. Embryos T, V, and Y, however, all have quotients too low for their respective ages. In other words the compression to which the nervous system is subjected is higher than normal and has resulted in too much differentiation.

The asymmetrical forms, U, W, and X, suggest that if a certain degree of compression fails to set in, or is not properly directed, the aberrant condition will be reflected in an asymmetrical distribution of the neural mass.

CONCLUSION

A comparative study of the illustrations in the plates and the values given in tables 1, 2, and 3 may, I think, be justly summarized by saying that our expectations in general have been

TABLE 2
Normal embryos

QUOTIENT	SOMITES	REMARKS
1.890	3	
1.672	2	
1.485	2	
1.313	4	
1.132	3	
1.0475	5	
1.017	5	
1.012	4	
1.0095	5	
0.968	6	
0.952	6	
0.949	6	
0.925	8	
0.920	7	
0.911	10	
0.903	7	
0.902	8	
0.9009	9	
0.887	9	
0.877	9	
0.871	10	
0.863	9	
0.851	10	
0.849	10	
0.847	10	
0.846	10	
0.842	10	
0.842	10	
0.837	9	
0.820	10	
0.820	10	
0.818	11	
0.812	11	
0.811	10	
0.800	11	
0.772	11	
0.737	13	Telescoped forms Plate 2 I
0.664	13	Telescoped forms Plate 2 J
0.649	15	Telescoped forms Plate 2 K
0.605	16	Telescoped forms Plate 2 L
0.592	15	Telescoped forms Plate 2 M
0.577	16	Telescoped forms Plate 2 N
0.573	17	Telescoped forms Plate 2 O
0.572	17	
0.572	16	

TABLE 3

QUOTIENT	EMBRYO	SOMITES	REMARKS	QUOTIENT
0.817	T	9	Asymmetrical	Too low
0.790	U	13	Asymmetrical	Too high
0.789	V	10	Overdifferentiated	Too low
0.787	W	13	Asymmetrical	Too high
0.768	X	13	Asymmetrical	Too high
0.541	Y	14	Overdifferentiated	Too low
0.522	Z	27	Overdifferentiated	

fulfilled. The relations postulated in advance between the neurocephalic quotients, on the one hand, and aberrancies, degrees of normal differentiation, and somitic increase on the other, are capable of being verified. The relation which at this time I wish to especially emphasize, is the association of a falling quotient with the multiplication of somites.

The quotient correctly understood is a measure of longitudinal compression. The somites are recognized as a measure of development. It follows, therefore, that the movements of the quotient are related in time and sense to the serial differentiation to the nervous system precisely in the manner in which they should be related, if compression in the longitudinal axis is a condition upon which the vesiculation of the nervous system

TABLE 4

SOMITES	CASES	QUOTIENT
2	2	1.579
3	2	1.511
4	2	1.163
5	3	1.025
6	3	0.956
7	2	0.912
8	2	0.914
9	5	0.873
10	11	0.845
11	4	0.801
13	2	0.701
15	2	0.621
16	3	0.585
17	2	0.573

depends. The assumption of a 'causal' connection between compression and the formation of the brain vesicles is therefore warranted.

The justice of this assumption appears still greater when we average the results for each stage of development dealt with in table 2. This was done and the outcome is given in table 4.

If now, with the aid of table 4 we construct a curve in which quotients are plotted along the ordinate and the number of somites along the abscissa, the relation between a falling quo-

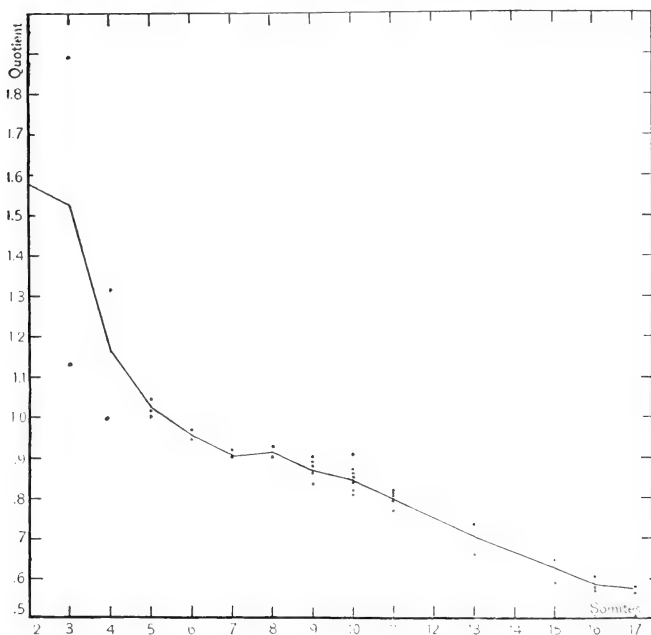


Fig. 8 Showing the relation between a falling neuro-cephalic quotient and somitic increase. The curve is constructed by joining the loci of the averages given in table 4. The dots indicate the loci of the individual quotients given in table 2.

tient and somitic increase exhibits itself in the most striking manner.

On the basis of this curve, figure 8, I infer that during the period of development considered, a rising state of longitudinal compression is one of the conditions determining the differentiation of a series of vesicles in the brain.

SUMMARY

1. Cranial flexure, although capable of explaining the lateral and ventral differentiations of the prospective second brain vesicle, is nevertheless not related to the general process of vesiculation in the manner in which it should be if the formation of vesicles were dependent upon flexure, as His maintained.

2. According to His, flexure depends on differential growth.

3. According to the results presented in the present paper, vesiculation also depends upon differential growth and precedes flexure.

4. Differential growth, to play a rôle in this connection, must be longitudinally effective. Such effectiveness is undemonstrable by the method of His.

5. Effectiveness in the long axis can be demonstrated by comparing the length of the embryonic head with that of the nervous system. The relation between these two measurements, within the arbitrary limits set by the cephalic rectangle, has been expressed in the form of a fraction.

6. This fraction, the Neuro-Cephalic Quotient, is derived by dividing half the perimeter of the nervous system into the head-length. It tells how many units of head-length are available for every unit of length in the nervous system.

7. The quotient is largest in the earliest stages, and decreases with the progress of development. It is inversely proportional to the number of somites, and, so far as can be determined in the absence of accurate modes of expression, with the degrees of differentiation exhibited by the nervous system. Telescoped forms, and those abnormally over-differentiated, have expectedly low quotients.

8. Despite the variability which inheres in developmental processes by their very nature, the relations between quotient on the one hand, and somites, or differentiation of the nervous system, on the other, are such as to warrant the conclusion that a rising state of compression in the longitudinal axis is one of the important conditions under which the vesiculation of the embryonic brain takes place.

PLATE 1

EXPLANATION OF FIGURES

Outlines of heads of diagrammatic 2 to 11 somite chick embryos referred to in table 1. The posterior line through the nervous system is the somitic base line. In embryo A the nervous system did not extend backward as far as the somitic tangent. In this case two base levels are indicated, one used to measure head-length, the other to determine the neural perimeter.

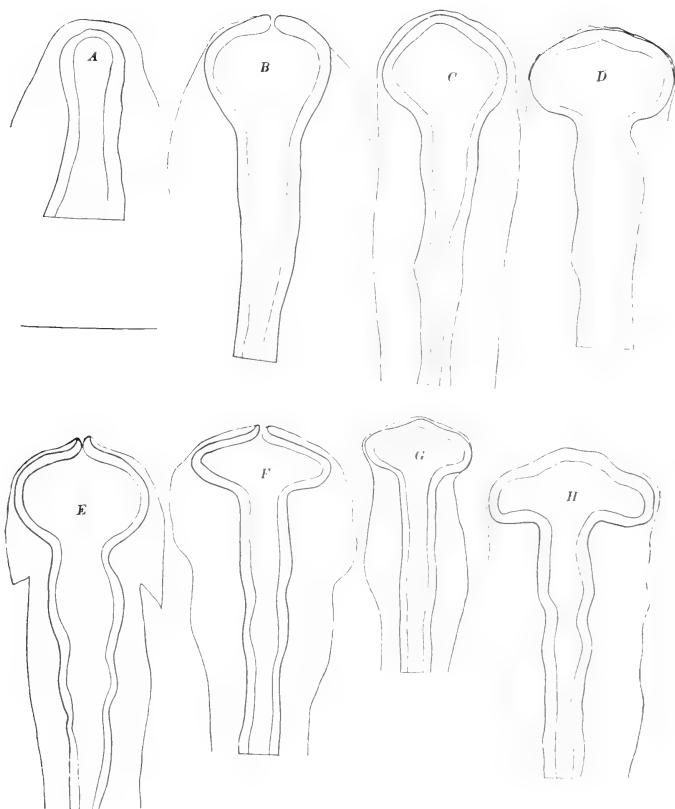


PLATE 2

EXPLANATION OF FIGURES

Telescoped forms with 13 to 17 somites and low quotients. Referred to in table 2.

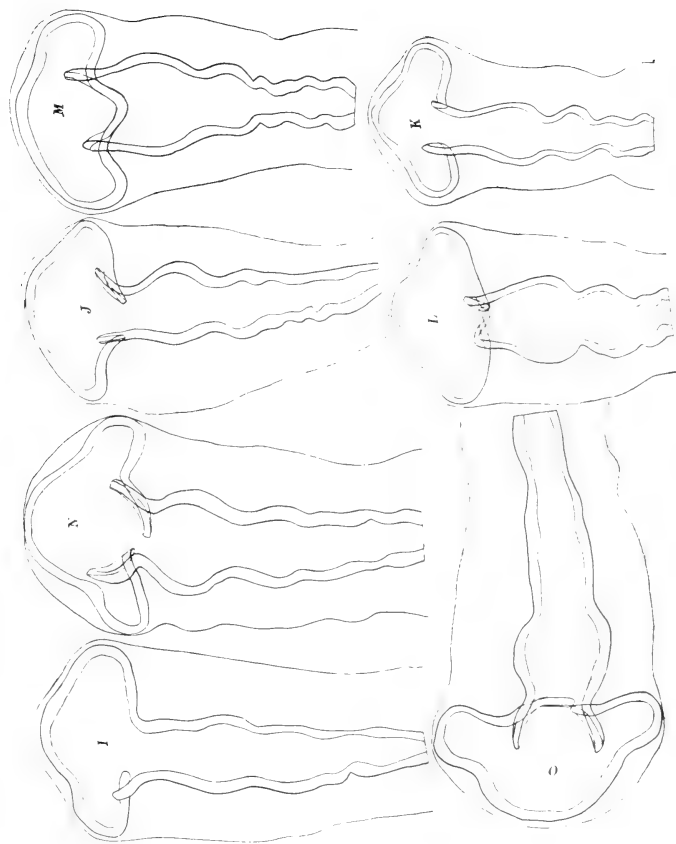
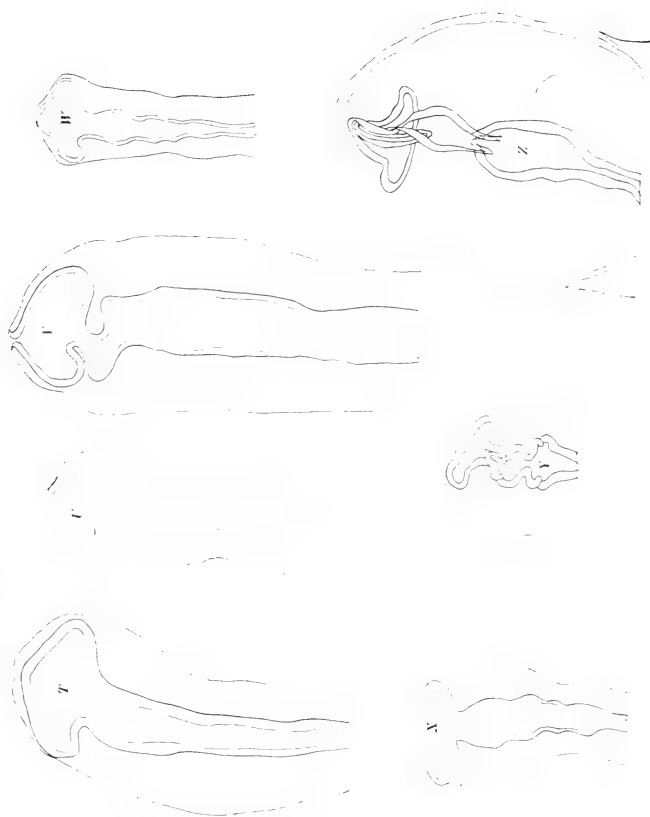


PLATE 3

EXPLANATION OF FIGURES

Aberrant forms. Embryos T, V, Y, and Z are over-differentiated. As shown in tables 2 and 3, T, V, and Y have quotients too low for their respective ages. The quotient normal for the 27 somite stages has not been determined; therefore no statement can be made regarding embryo Z.

Embryos U, W, and X, have quotients too high for their respective ages, and suggest that compression must reach a certain magnitude and moreover be properly directed in order that the neural mass may distribute itself symmetrically.



THE CHOROID PLEXUS WITH SPECIAL REFERENCE TO INTERSTITIAL GRANULAR CELLS

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TEN FIGURES

During a comparative study of the structure of the choroid plexus in various mammals, in association with Dr. Harvey Cushing at the Hunterian Laboratory, Johns Hopkins University, I observed the presence of very prominent interstitial granular cells in the choroid plexus of the ox. They are especially numerous in the ox and are present to a lesser degree in the sheep and swine. I have failed to find any reference to these cells in the literature on the choroid plexus, and it is primarily to report them that this paper is submitted. I have included, as well, my observations on the structure of the epithelial layer of cells, in view of their now generally accepted connection with the secretion of cerebro-spinal fluid.

GENERAL ARCHITECTURE

The choroid plexus of the ox is similar in structure to that of other mammals, as generally described. Tufts of blood vessels varying in size and thickness of the connective tissue coats are seen covered by a single layer of cuboidal cells.

The various elements making up the choroid plexus are clearly differentiated in tissues fixed in formalin-bichromate solution and stained in Van Gieson's. The yellowish-brown stained surface cuboidal cells are sharply demarcated from the red stained connective tissue which surrounds the blood vessels. In some tufts the connective tissue coats which form the walls of the blood vessels measure 0.2 mm. in thickness, while in others they form

only a thin membrane between the vessel lumina and the surface cuboidal cells. Much variation exists also in the size of the lumina (fig. 1).

Generally speaking, the vessels of the choroid plexus may be divided into two groups: 1) The vessels with the thick walls possess as a rule narrow lumina and have the same structure in general as do small arteries. Weigert's stain shows a well developed tunica elastica interna, which in most instances is cor-

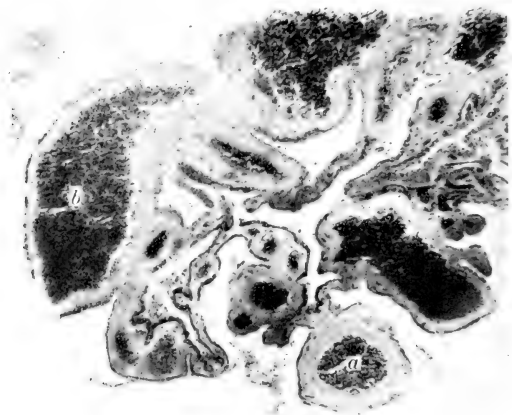


Fig. 1 Choroid plexus of the ox. *a*, artery; *b*, vein or sinus. Microphotograph, oc. 10, obj. 16 mm., B. & L. Tech: Bensley's alcohol bichloride bichromate solution, haematoxylin and eosin.

rugated. Fine, waving elastic fibers may be seen in the tunica media, which is well developed. The fibers may be further traced to the cuboidal cells and are frequently seen to form a basement membrane for these cells. 2) The second group of vessels are those with comparatively thin walls and wide lumina. These may be regarded as veins or sinuses. The walls are composed almost entirely of white fibrous connective tissue and cannot be differentiated into intima, media, and adventia, but are similar in structure throughout. Only here and there are

seen fine elastic fibers. No muscular walls can be made out in this second group.

The walls contain numerous small vessels and capillaries (*Vasa vasorum*) of varying calibres. They are rich also in nuclear elements, such as endothelial cells, connective tissue cells and interstitial granule cells (fig. 1). A discussion of these interstitial granule cells follows that of the cuboidal surface cells.

EPITHELIAL CELLS

The epithelium forms a single layer of cuboidal cells. This observation agrees with those of Meek ('07) and others who describe a single layer of cells. Luschka ('55), Findlay ('89) and Kölliker ('96), however, state that several layers are present. The cells on some tufts are more or less flattened while on other tufts they are found to be elongated. This condition depends upon the degree of distention of the vessels. Generally the epithelial layer presents on the ventricular surface an even, uninterrupted surface, so closely and evenly are the cells arranged. Tufts are present, however, where slight indentations projecting basalward between the cells are seen. Thus the rounded free ends of the cells, in cross sections, give the epithelial layer a corrugated appearance. Frequently cells are seen with bipartate free ends.

In the ordinary fixations and stains the cytoplasm in the majority of the epithelial cells stains evenly and compactly throughout. A very finely granular substance is distributed throughout this cytoplasm. No intracellular net work is seen. Vacuoles are frequently present. The nuclei vary somewhat in form. As a rule, they are spherical. In the more or less flattened epithelium they are oval in outline, with their long axes parallel to the surface of the tufts. Much chromatin is present in the nucleus although it does not stain solidly throughout, as is the case in many gland cells. Several large chromatin masses, as a rule, are seen in each nucleus surrounded by numerous finer granules. One or two nucleoli may be seen. These, however, are difficult to differentiate in the ordinary stains from the

larger chromatin masses. The nuclei occupy the central portion of the cells, perhaps somewhat more basalward than towards the free surface. Examination of many choroid plexuses does not reveal any marked variation to this position. I did not observe a nucleus compressed against the basal end of the cell, as is seen in many gland cells.

For a more detailed study of the surface epithelium, choroid tissues were fixed in Bensley's alcohol bichloride bichromate solution and formalin bichromate solution. Sections were stained with iron haematoxylin, copper chromohaematoxylin, Bensley's ('11) neutral gentian and safranin acid violet. In all these stains the epithelium stands out prominently. The cell membrane on the surface is seen as a relatively thick line, at times it appears as a double contoured line. No striations are seen in this cell membrane. After some fixations, where there is evidence that the process has not been perfect, the cell membrane appears as a thick margin, still retaining the original shape of the cell while the cytoplasm is shrunken away from it. In such preparations, the impression is given that these cells have thick, more or less unyielding membranes. No cement substance is present between the cells. Secretion granules, such as are seen in the pancreas, parotid, lachrymal, gastric and other glands, are not seen in the epithelial cells of the choroid plexus. However, after staining with neutral gentian or safranin acid violet, one finds cells which possess a few deeply stained granules that simulate in structure and staining characteristics the secretion granules of the above named gland cells. Such cells are not numerous, and the granules are only sparsely present, not more than one-half dozen have been counted in each cell. The nature of these granules has not been determined. They may represent nuclear substance within the cytoplasm. Occasionally one observes as well, large bodies near the nucleus which take the nuclear stain deeply. These may be interpreted as para-nuclei and have been observed in various cells.

The epithelial cells possess numerous vacuoles and canaliculi. Others have observed vacuoles in the epithelial cells of the choroid plexus. Meek found fat globules in these cells in the rabbit,

which in prepared sections, owing to the dissolving action of the various preparation fluids, appeared as vacuoles. He proved that these were due to the former presence of fat, by staining with Sudan III choroid cells which had not been subjected to the dissolving action of alcohol and xylol. Globules, or vacuoles, have been observed in this epithelium by Luschka, Findlay, Studnicka, and Galeotti, which, according to these observers, are evidences of the vesicular type of secretion. On the other hand, Pettit and Girard are prone to regard the vacuoles as abnormal structures due to mechanical injuries or post mortem changes.

Numerous intracellular spaces were seen in my preparations notwithstanding that the greatest care was exercised to avoid mechanical injury to the cells. The tissues were removed and fixed almost instantly after the animal had been slaughtered, so that no postmortem changes could have occurred. These spaces are in the form of globules or vacuoles and canaliculi. The vacuoles may be seen in any part of the cell, either at the base or the free margin. They may be round or oval. Occasionally a row of these oval vacuoles are seen at the base of the cell.

The intracellular canalicular apparatus of Holmgren ('02), Bensley ('10) and others, may be seen in the cytoplasm as branching canals which frequently can be traced to the nucleus and partially encompassing it. They are entirely intracellular, as the branches terminate within the cytoplasm (fig. 3a).

That the finer vacuoles are cross sections of these canals is probable. Whether they bear any relation to the secretory activity of the gland, I am unable to say at the present time. That the larger vacuoles do not represent spaces remaining from dissolved fat globules is certain, for I have been unable to demonstrate the presence of such fat globules in the epithelium of the choroid plexus of the ox (figs. 3, *b*, *c*).

The description so far given for the epithelial cells is confined to those which are generally observed. Studies of numerous choroid plexuses, however, show various types of cells. In addition to those already described, one sees in fixed preparations

cells whose cytoplasmic area is composed almost entirely of vacuoles. The cytoplasm is manifested only between the vacuoles, forming a network surrounding the spaces. In another type, the entire cell is seen to be enlarged. It may be two or three times as large as the general type. It bulges out and is rounded as a consequence of its contents (fig. 2). This type shows that a confluence of vacuoles has taken place. This cell stains faintly because of the relative decrease in the cytoplasm.

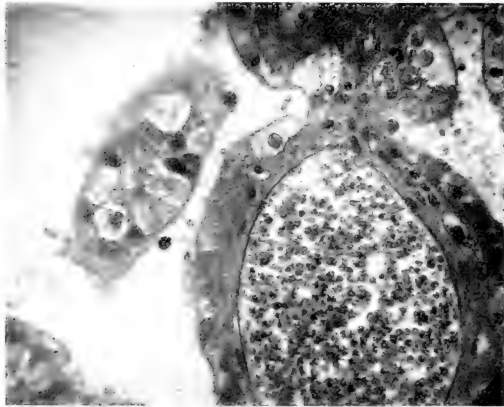


Fig. 2 Choroid plexus of the ox. Two types of epithelial cells are seen in the section: large, faintly staining, distended cells; and small deeply staining cells, which is the type usually making up the epithelium. Microphotograph, oc. 10, obj. 4 mm., B. & L. Tech.: Same as 1.

Only at the base of the cell is seen a narrow ragged zone of compact cytoplasm. In these large vacuolated cells, no changes are observed, as a rule, in the form and position of the nuclei, which are generally centrally located; however, I have observed the nuclei in the apices of many of them. The large bulging vacuolated cells may be seen between two smaller cells whose cytoplasm is compact. Again they may constitute the majority of cells on a particular tuft or villus.

That these various cell types belong to the same category and that they represent different secretion stages of the same cell type is evident.

From the study of these cells in fixed sections, one is constrained to believe that the secretion of the epithelial cells of the choroid plexus is a vesicular one. The secretion substance makes its appearance at the base of the cell as minute vesicles. Later they are seen throughout the cytoplasm. A confluence of these vesicles takes place to form large vesicles. At the same time the cell enlarges, becomes rounded and bulges out between the resting cells. Then the secretion passes out into the ventricles without a break in the continuity of the cell membrane.



Fig. 3 Three cells, selected, showing canals and vacuoles within the cytoplasm. *a*, intracellular canaliculi; *b* and *c*, vacuoles. Drawing somewhat diagrammatic, oc. 10, obj. 1.9 mm., oil immersion, B. & L. Tech.: Bensley's alcohol bichloride bichromate solution, neutral gentian.

That a continuous secretion is taking place is apparent from the fact that all the various secretory stages of these cells may be seen in one choroid plexus. One finds, however, some tissues in which the vacuolated cells are much more prominently present than they are in others. As a rule, the epithelium is of the general non-vacuolated cuboidal type which is described first.

The processes of secretion in the epithelial cells are in no way comparable to those of the duct gland cells,—salivary, pancreas, lachrymal glands. Secretion granules, the antecedents of these granules, and nuclear changes as seen in other gland cells are not demonstrable in the epithelium of the choroid plexus. The secretion substance of the latter makes its appearance as minute droplets within the cytoplasm and no antecedent secretion substances have been observed. A somewhat similar

method of secretion has been observed in the kidney by Gurlitsch and others. In other respects the secretion methods of the two organs,—choroid and kidney—are similar. In both large amounts of fluid are secreted (excreted) with but relatively little changes in the cells concerned in the secretion. The latter differs from the former in that no foreign substances, such as injected indigocarmine, potassium ferrocyanide, iron-ammonium citrate, prussian blue, have been observed to pass through the choroid epithelium into the ventricles. Experiments were performed on dogs by Prof. S. A. Matthews and myself in order to find some substance that, when injected into the blood, could be detected microchemically in the epithelium of the choroid. Our results will be published later.

The action of the choroid cells may be compared also, to that of the endothelial cells of blood vessels in the formation of lymph, providing we accept the theory that physical processes alone—filtration, diffusion and osmosis, do not explain all the phenomenon of lymph secretion, but that the endothelial cells are concerned in the secretion of lymph, in which case a large amount of fluid is produced without any distinct observable histological changes on the part of these endothelial cells engaged in lymph secretion.

Meek found that with an increase of cerebrospinal fluid following muscarin injection, the epithelial cells had increased in height and certain clear spaces formed in the apical ends of the cells. His observations corroborated in a way the observations of Pettit and Girard ('02).

I have not confirmed, in the opossum, the observations of Meek in this respect, but my observations are limited to a few adult animals. One must presume that the choroid plexus has an autonomic innervation like the salivary glands, etc., in order to secure this phenomenon. I have not demonstrated to my own satisfaction, either by the silver method or by vital methylene blue staining, that the cells of the choroid plexus are innervated.

I am inclined to hold that the choroid plexus cells and their activity in the secretion of cerebro-spinal fluid belong to the

category of endothelial cells and cannot be compared to duct gland cells derived from epithelium.

Definite intercellular spaces are also observed. These may be in the form of mere indentations between the surface ends of the cells or a definite cleavage may be seen reaching to the basal end of the cell, where it frequently appears continuous with other canal-like structures which enter the deeper connective tissue walls (fig. 4). Occasionally the intercellular spaces are globular in outline. These observations do not agree with those of Meek and others, who hold that the epithelial cells are so closely appressed that intercellular spaces do not normally occur and that

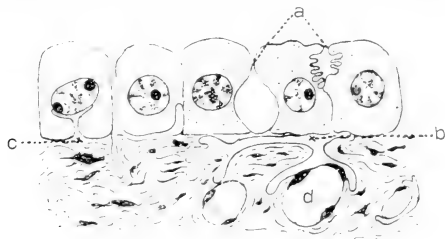


Fig. 4 Composite drawing of cells showing: *a*, intercellular canals; *b* and *c*, sub-cellular and interstitial canals; *d*, blood vessel. Drawing somewhat diagrammatic, oc. 10, obj. 1.9 mm., oil immersion, B. & L. Tech.: Same as 3.

when they are seen, they are due to faulty technique. Studnicka ('00), on the other hand, holds that these spaces are seen in the choroid plexus of the shark.

While I have not observed these intercellular and sub-cellular canals in living tissue, I am not convinced that they are always to be regarded as artifacts. The frequency with which they are seen, the variations observed in their outlines, point to the fact that they are normally present. The canals are frequently seen to continue as such in the connective tissue walls and apparently communicate with lymphatics. To maintain that they are normally present does not seem inconsistent when one compares the choroid plexus with other serous endothelial cells. In my opinion, these spaces can be readily compared to the stomata

described by v. Reklinghausen, Klein, Dogiel, and others between the endothelial cells of the peritoneum and by Ludwig and Dybkowsky in the pleura.

The silver precipitation methods were utilized in the study of these intercellular canals. A heavy black precipitation filling the entire epithelial cell occurs when the tissue is fixed in Kopsch's fluid. Thus when viewed through the low power of the microscope, this deposit in all the surface cells forms a black border around the choroid tufts. Projecting from the basal margin of this black border (the epithelial cells) into the connective tissue are seen numerous canal-like deposits which suggest the intercellular and subcellular canals. However, further observation is essential before I can come to any definite conclusion in this particular. The very heavy deposit of silver within the cells makes the observations difficult and one is not always sure as to whether he is dealing with definite tissue structures or with artefacts.

Further investigation is essential before one can state just what the function of these intercellular canals is. When compared again with the generally accepted function of peritoneal stomata, it is suggested that these canals may be absorptive in character, that is, the cerebro-spinal fluid may pass back into the circulation through them. If such is true, the choroid plexus then may be regarded as both a secretory organ and an absorption organ. On the other hand, the presence of the canals suggests another possibility, and that is, that they may function directly in the production of cerebro-spinal fluid. Should we accept the mechanical theory of secretion, it would not be fantastical to suppose that much of the fluid may be regarded as a transudate passing directly from the connective tissue surrounding the lymphatics and blood capillaries through these small sub- and inter-cellular canals directly into the ventricles, without passing through the epithelial cells. According to this hypothesis, the epithelial cells of the choroid plexus alone may not be concerned in the production of cerebro-spinal fluid. In view of the fact that large amounts of cerebro-spinal fluid may be secreted in a short time (200 to 300 cc. and possibly more in 24

hours in the human, Cushing '14), one might naturally expect to see more histological evidence of secretory activity on the part of the epithelial cells, providing these cells are solely concerned in the production of the fluid. Another observation that suggests this second hypothesis is that in the choroid plexus of the ox, numerous mast cells apparently pass directly from the blood vessels through the connective tissue wall and between the epithelial cells into the ventricles.

Against this view that the cerebro-spinal fluid may be regarded in part as a transudate is the fact that it differs from lymph,

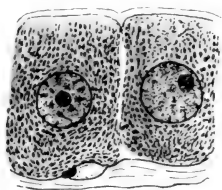


Fig. 5 Mitochondria in the epithelial cells. Drawing, oc. 10, obj. 1.9 mm., oil immersion, projection apparatus. Tech.: Bensley's acetic osmic bichromate solution, anilin fuchsin methyl green.

plasma, tissue juices and serous cavity fluids in the relative amounts of protein substance. Cerebro-spinal fluid is almost free from protein, while the other fluids contain much more.

That the choroid plexus is responsible for the formation of cerebro-spinal fluid was suggested by Faivre a long time ago. Luschka, a little later, agreed with him, and practically all observers since, including Pettit and Girard, Studnicka, Galeotti ('97), Carazzani, Meek, Mott ('10), Goldmann ('13) and Cushing, regard this structure as fundamental in the secretion of cerebro-spinal fluid. Cushing saw drops of the fluid exuding from this tissue. Goldman noted the extrusion of glycogen in the form of globules from the cells. Weed, in connection with others, suggests a dual origin of cerebro-spinal fluid—from the choroid plexus and from the perivascular systems of the nervous tissue.

Certainly much more work must still be done in order to solve the method of secretion which takes place in the choroid plexus.

When the tissue is prepared by Bensley's acetic osmic bichromate, anilin fuchsin, Methyl green method, the epithelial cells show numerous mitochondria. These are in the form of very short bacillus-like rods and tend in a way to arrange themselves in irregular rows, reaching from the base of the cell to the summit. Of course these rows are by no means so definite and clean cut as I ('16) have observed in the duct cells of the lachrymal glands (fig. 5). In the vacuolated cells, the mitochondria are seen in the cytoplasmic reticulum between the vacuoles.

INTERSTITIAL GRANULAR CELLS

This type of cell is characterized by the presence of numerous large granules which completely fill the cell. The nucleus is as a rule completely obscured by these granules which simulate in form and staining characteristics the secretion granules of gland cells.

These granular interstitial cells were found to be present without exception in the choroid plexus of the ox, but varied in number with different animals. In some, they were especially numerous, while in others only a few were found. Between these two extremes there was every gradation. In the sheep and swine they are not so numerous. In the choroid plexus of other animals examined—rabbit, guinea pig, dog, and human—these cells have not been observed.

The following description of the interstitial granule cells will be confined to those observed in the choroid plexus of the ox. The cells vary in size and shape. The average size is 20 micra. The majority are more or less spherical or oval in outline. Many, however, are angular, others are elongated or irregular and possess processes extending from the cells. They are situated in the connective tissue walls of the vascular tufts between the vessels' lumina and the surface epithelium. In some fields (low power microscope), as many as 30 of these cells have been counted (figs. 6, 7, and 8).

The thickest connective tissue walls, those surrounding the arteries, contain the largest number of these cells, which are found at varying distances from the lumina of the vessels. Some are seen in close contact with the endothelial cells of the vessels and in some instances, processes of the granular intersti-

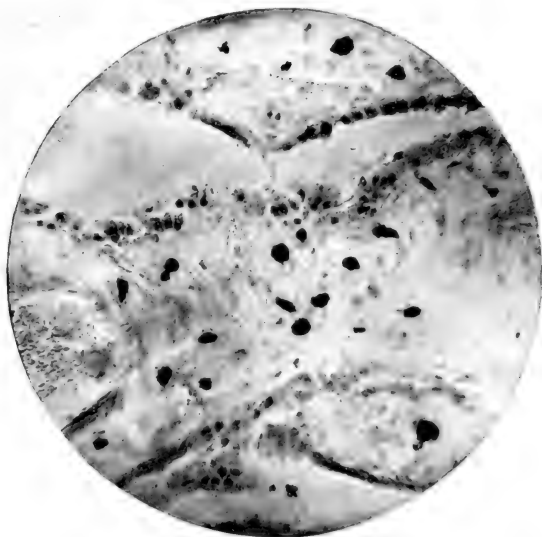


Fig. 6 Stroma of choroid plexus of ox. The numerous interstitial granular cells—mast cells—are deeply stained. Microphotograph, oc. 10, obj. 4 mm. B. & L. Tech.: Same as 3.

tial cells are seen to project between two endothelial cells into the lumen. The other extreme is seen where processes of these cells lying directly under the epithelial layer of the choroid plexus project upward between two epithelial cells into the ventricle. Between these two extremes the granule cells are seen in the connective tissue at different levels. None of these cells have been observed lying free either within the vessel lumina or on the ventricular surface of the choroid plexus.

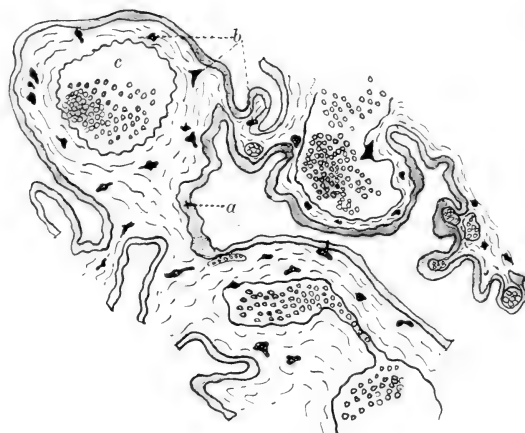


Fig. 7 Interstitial granular cells—mast cells—in the connective tissue wall. *a*, Surface epithelium; *b*, The deeply stained interstitial granular cells—mast cells—are seen at varying distances from the lumina of vessels. Some are directly underneath the epithelium with processes projecting between the epithelial cells. Others are seen directly underneath the endothelium of vessels; *c*, artery. Drawing, oc. 10, obj. 16 mm., B. & L. projection apparatus. Tech.: Bensley's alcohol bichloride bichromate solution, Unna's polychrome methylene blue.



Fig. 8 Four selected interstitial granular cells—mast cells—showing variation in form. Drawing, oc. 10, obj. 16 mm., B. & L. projection apparatus. Tech.: Same as 7.

The cells are readily seen in both fresh tissue mounted in serum or salt solution and in fixed preparations. Bensley's solution—equal parts of (a) saturated solution of mercuric chloride in 95 per cent alcohol and (b) 2.5 per cent aqueous solution of potassium bichromate—proved to be the most satisfactory fixing solution for the granular cells. They are fairly well fixed in alcohol. Fluids containing much acid destroy the granules, consequently Zenker's solution cannot be used. In Bensley's acetic osmic bichromate solution, the granules are only partially conserved. This fact, however, proves of great value because then the other constituents of the cell can be made out,—nucleus and intergranular substance.

The stains first utilized for the study of these cells were Bensley's neutral gentian and neutral safranin. The granules in these dyes stain deeply and hold on to the stain with much tenacity. Even after the section as a whole has been differentiated in alcohol-clove oil to the extent that practically all the stain has been removed, the granule cells remain deeply stained. And so numerous are these granules within the cell, as a rule, that the entire cell appears as one deeply stained mass. It is only after extended differentiation or in the processes of the elongated cells that the individual granules can be made out.

In these stains the nuclei in most cells are completely obscured by the deeply stained granules. Only in those cells where the knife has passed through the nucleus or in the smaller cells where only few granules are seen around the nucleus is the latter seen. It occupies a central position. It is oval in outline and vesicular,—thus staining faintly. Only a small amount of chromatin is present which is distributed throughout the nucleus appearing as fine irregular clumps. No nucleolus is observed. When these nuclei are seen through the low power of the microscope they appear as transparent or faintly opaque areas in the center of the cells surrounded by the deeply stained granules (fig. 9, *a*).

The presence of so many of these large round or oval mononuclear cells possessing numerous granules which simulate, both in size and staining characteristics, secretion granules suggested

at first the possibility of the choroid plexus containing some type of endocrinous gland (fig. 6).

Extracts of the choroid plexus were made in the usual manner and injected intravenously into a dog. Outside of the usual depressor reaction,—a frequent phenomenon accompanying injections of animal tissues,—no specific or particular reaction was observed. Dixon and Halliburton ('13) on the other hand claim that choroid extract when injected intravenously increase markedly the flow of cerebro-spinal fluid. Our observations, how-

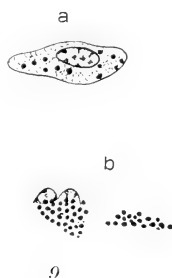


Fig. 9 *a*, Interstitial granular cell, in which the large basophilic granules are not conserved. The mono-nuclear character of the cell is clearly seen. Note the fine intergranular network of cytoplasm. *b*, Broken mast cells, the granules of which are fuchsinophilic. Drawings, oc. 10, obj. 1.9 mm., oil immersion, B. & L. Tech.: Same as 5.

ever, were confined only to one experiment and consequently will not warrant any conclusion on this phase of the subject.

The probability that these interstitial granular cells are mast cells early suggested itself, especially after other stains had been used. However, I have not seen them so constantly and prominently present in any other tissue. Their presence in such large numbers may suggest that they have some function other than that generally ascribed to mast cells. However, our knowledge of the nature and function of mast cells is very superficial. Numerous theories have been advanced regarding the rôle they play in animal life. Many hold that they are in a way unicellular glands concerned in some type of secretion. If such be the

case, one may still regard these interstitial granular cells of the choroid plexus as having some unknown secretory function. At any rate, it was later determined that they possess the same morphological and staining characteristics as the so-called 'histiogenen Mastzellen' of Ehrlich and others. In view of these facts, the interstitial granular cells of the choroid may be regarded, for the present, at least, as belonging to this category, although their particular function here is unknown.

Since Waldeyer ('75) first described a particular group of connective tissue cells possessing granules, to which the term 'plasma cells' was applied, and later Ehrlich ('77) and his assistant Westphal ('80) recognized a still more limited group whose large granules showed a strong affinity for basic dyes and to which the term 'mast cells' was applied, much interest has been shown in these interstitial granular cells.

In the literature on this subject, mast cells are generally described as interstitial cells more or less round in contour, the cytoplasm of which is made up of large granules which have a marked affinity for basic stains, Mallory ('14), Schäfer ('12), Rauber Kopsch ('06). Most authors fail to differentiate between the two types first recognized by Ehrlich and Westphal, who referred to them as 'mastleucocyten' and 'Histogenen mastzellen.' Some writers describe the former and some the latter, when a definition of mast cells is made.

Regarding the two types, Prenant, Bouin, Maillard ('04) state: "Les cellules à granulations basophiles du sang sont les mêmes que les cellules nutritives que nous retrouverons dans les tissu conjonctif sous le nom de Mastzellen ou cellules-engrais." The two cell types were recognized by Pappenheim ('01) who discusses the origin of each, and by Maximow ('06) who states that the relation of the two types is not clear.

The distribution of mast cells, according to many investigators, is wide. This is true not only of the distribution in the individual, but also throughout the various orders of animal life. They have been described both in invertebrates, such as the cellules mucoides, described by Guenot in 'Gastropodes Pulmones' and in every order of vertebrates. It was the opin-

ion of early investigators that mast cells were found in the latter only in batrachians, where in the Urodeles they were seen in enormous numbers. Now it is generally conceded that they are found in all vertebrates. They have been described in triton, frog, turtle, rabbit, calf, man, and in fact, all species of vertebrates.

In the individual body they are distributed as follows: In the blood-basophilic leucocytes; connective tissue mast cells have been described in the connective tissue layers of the skin and mucous membrane. They are numerous in the tongue, in the septa of various glands, in the lungs. Arnold ('14) found that mast cells are greatly increased in numbers in the frog's tongue after induced passive congestion. Müncheimer ('95) saw them in the testes of horse, rat and pig. He failed to note them in these organs of the deer, sheep, dog and rabbit. Korybutt Daskiewicz ('78) describes them along the nerve fibers in the frog. McKibben ('14) states that they may readily be mistaken for nerve cells as observed in the nasal region and meninges of *Necturus*. They are found in bone marrow, adipose tissue, along blood vessels, in newly formed connective tissue. These mast cells make their appearance early in embryonic life. They have been observed in the 9-day chick and in early calf embryos. These cells are much more clearly seen, as a rule, in the embryo than in later life.

I have failed to observe in the literature on this subject reference to these large, prominent interstitial granular cells—mast cells—in the choroid plexus of the larger mammals. Haeckel ('59) refers to certain interstitial cells in the choroid plexus of embryonal mouse and dog, which, according to him are similar to those seen by Schultze ('56) in the gelatinous connective tissue of tunicates and medusae. Haeckel considers these wandering cells and, according to him, they are filled with minute globules of fat.

I have stained the choroid plexus of the ox with a view of determining the presence of fat in the mast cells. Sudan III, Scharlach Rot—in alcohol or as Herxheimer's stain, and the Nile blues were used. So far, I have failed to demonstrate the

constant or prominent presence of fat in these mast cells. Only rarely are fat droplets seen. Hence the mast cells in the choroid plexus of the ox differ from those cells described by Haeckel. They are also different from the other mast cells of the connective tissue type in which fat and lipoid substance have been found, as claimed by Pappenheim, Posner, Lombardo and others. Huguenin ('12) saw numerous lipoid granules in mast cells in a case of status lymphaticus. According to Ciaccio ('13), lipoid and fat are found in these cells only under pathological conditions. Flemming ('71, '76, '79) and Hammar ('95) were of the opinion that fat was not deposited in mast cells. Certainly fat is not demonstrable as a constant constituent of the mast cells of the choroid plexus.

Reaction to mucin stains. One of the first stains used in the study of these cells was muchaematein, in which the granules stained a deep blue. No other elements in the tissue were stained. The presence of so many round or oval cells specifically stained in muchaematein or mucicarmin was another factor that suggested a glandular secretion phase to these interstitial cells. Of course, the presence of mucin naturally suggested itself. I ('16) have shown, however, that secretion granules—lachrymal gland—do stain specifically in these mucin stains, although we have no evidence that mucin is secreted by the lachrymal gland. Others have observed that the granules in mast cells are stained with mucous stain. Raudnitz ('83) held that because of this reaction, the granules represent a stage in mucinous degeneration of the cell. Hoyer ('90) held that the granules were of a mucinous nature. Schaffer concludes that as the mast cells take the same stain as do cartilage cells, the former contain a chondroitin sulphuric acid substance. On the other hand, Pappenheim, Schwenter, Trachsler and others held that the granules of mast cells have no relation to mucin. Ehrlich observed that the granules of mast cells are much more resistant to water than are mucous granules.

An interesting phenomenon in the study of muchaematein stained mast cells is the variability in the intensity of the staining reaction of the granules. Some cells take the stain much

more deeply than do others. One frequently secures choroid plexus in which the cells as a whole stain much more quickly and deeply than do the similar cells in other choroid tissue. This variety in intensity of staining with muchameatein may represent various stages in the formation of granules. No other elements in the cell are stained. Preparations according to this method are of great value in the study of the individual granules. These are seen to fill the entire cell and where processes are present, the granules extend into them to the very ends of the processes. They vary in size.

Reaction to basic stains. When stained in methylene blue, toluidin blue, methyl green, etc., the granules are deeply stained. They hold these stains with such tenacity that when the sections are differentiated in alcohol, practically all the stain may be removed from the section before there is any perceptible loss of stain from the granules. The nucleus, when seen at all, occupies a central position in the cell and appears as an opaque or semitranslucent structure.

Iron haematoxylin and copper haematoxylin stains. The granules do not retain these stains after differentiation. In fact, the mast cells are among the first of the structures to give up the stain during the process of differentiation. The granules of the mast cells differ in this respect from secretion granules of many serous cells. The latter retain both stains deeply after practically all the iron and copper reactions have been removed from the section as a result of the differentiation.

Polychrome methylene blue stain. Here the granules stain metachromatically—a deep violet, while the remainder of the choroid tissue in general is only very faintly stained. The epithelial layer is tinged green. Here, as observed in other stains, the granules in the mast cells are so densely stained that the entire cell appears as a dark violet mass simulating an artefact. Prolonged differentiation in alcohol reveals the characteristics of the individual granules, especially in those cells where the granules are not so numerous. In many cells a translucent central area is observed which marks the position of the unstained nucleus.

In this fixation (Bensley's) and stain, the nucleus is never definitely seen because of the numerous granules that obscure it.

Alcohol fixation. The granules are not so well conserved in 70 per cent alcohol, although according to many observers, alcohol is one of the best fixatives for mast cells. This method of fixation, however, has its value, as the nuclei of the cells are plainly seen, owing to the fact that the granules are not well preserved. The nucleus is stained green (Polychrome methylene blue). It is very vesicular and is as a rule oval in outline. No nucleolus is present, as was also observed by Dantchakoff ('98), who states that no nucleolus is present in the nucleus of mast cells. Variable sized chromatin granules are present. As a rule one to four or five large chromatin masses, one to two micra in diameter, are seen in each cell. Interspersed among these chromatin granules is the fine chromatin dust. The chromatin is only sparsely present, which accounts for the vesicular appearance of the nucleus. The granules of the mast cells are almost totally destroyed when fixed in Bensley's acetic osmic bichromate solution. Here the nucleus can be readily observed. I was unable to demonstrate the presence of mitochondria in these affected cells after fixing in the above solution and staining with anilin acid fuchsin, methyl green. Occasionally, however, mast cell granules were seen which, instead of being stained green by the methyl green, were stained deeply red by the fuchsin (fig. 9, *b*).

When the tissue is prepared according to the method utilized by Cowdry ('16) and Scott ('16) for study of mitochondria, the granules of the mast cells are well preserved and are stained deeply green in contrast to the red stained mitochondria so abundantly present in the surface epithelium. I did not observe mitochondria within the mast cells. Had any of these minute fuchsinophilic granules been present, they should have been observed readily in contrast to the deep green stained mast cell granules. This observation is corroborated later on in the vital stains.

VITAL STAINING

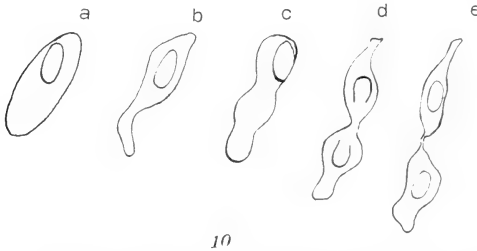
Choroid tissue was obtained by gently separating the plexus from its attachment in the ventricle immediately after the animal was slaughtered. Small bits of the choroid were then placed in the following solutions: Neutral red, one part in 15,000 of isotonic salt solution; new methylene blue, one part in 10,000 of isotonic salt solution; janus green, Metz, 1 to 15,000; polychrome methylene blue, Unna, 1 to 15,000; and pyronin, 1 to 1,000. Small pieces were removed from each of these solutions, mounted in the respective solutions and studied under the microscope. The remaining tissues were fixed in ammonium molybdate solution. The following observations were made:

Neutral red stain. The choroid plexus is rapidly stained with this dye. Macroscopically, the entire tissue is stained deeply red. Microscopic examination reveals the following characteristics: The surface epithelium is stained deeply red, the stain is confined to the cytoplasm and is diffuse throughout. The numerous, minute, highly refractive granules which were observed in fresh, unstained tissue, stand out prominently in the diffusely red stained cytoplasm. The nucleus which is unstained is spherical and occupies a central position in the cell.

Numerous mast cells are seen, the granules of which stain rapidly and deeply red. The large oval nuclei of the mast cells remain unaffected by the stain. As a rule, they are much more readily seen in this preparation than in the permanent preparations. Contrary to the observations of Arnold, I noted some activity on the part of these mast cells when mounted in serum and enclosed within a stage incubator. One cell was seen to completely divide within the period of one and one-half hours. This particular cell, which was oval in outline at first, was seen to elongate itself by processes extending from both ends (fig. 10). The formation of new granules in the processes was concurrent with the development of the processes. With the elongation of the cell, a constriction occurred in its central portion, which continued until the cell was completely divided, and with the division of the cell, nuclei appeared in both halves. Of course it was

impossible to observe in this stain the method of nuclear division. Arnold saw no changes in the form and position of mast cells within a period of from twenty-four to thirty-six hours. Kanthack and Hardy held that the 'Histiogenen' mast cells are stationary. While Lowenthal, Maximow, Pappenheim and Weidenrich ascribed to them an amoeboid movement. Maximow saw alterations in the form of mast cells in inflammatory tissue.

In numerous other cells, processes were observed to extend out from the cell bodies and to work themselves between the con-



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Fig. 10 Outline of changes observed in a living interstitial granule cell—mast cell—during division. The time required was one hour and fifty-five minutes. *a*, 4.35 p.m.; *b*, 5.05 p.m., *c*, 5.50 p.m., *d*, 6.10 p.m., *e*, 6.30 p.m. Drawing, oc. 1-, obj. 1.9 mm., oil immersion, B. & L. Tech.: Fresh choroid tissue mounted in serum, vital staining with neutral red, electric incubator for microscopic stage, temperature, 98.5°.

nective tissue elements. Some of the processes developed within one-half hour. The development of the processes was characterized, as a rule, by a continuous out-flowing of granules from the cell body. Granules were seen, however, to develop in the processes some distance from other granules, thus apparently having their origin directly from homogeneous cytoplasm. In attempting to determine the origin of these granules, I have come to no conclusion. In many instances the granules appeared to divide. However, as noted, granules seemed to make their appearance directly from homogeneous cytoplasm. Many observers hold that the granules are derived from the

nucleus. Downey states that the chromatic substance comes from the nucleus, through its wall, and then comes in contact with the primary granules of the cytoplasm, after which the basic and metachromatic staining of the granules follow. My observations show that the granules may form in the processes some distance from the nucleus. Just what relation they bear to the nucleus is impossible to state. The fact that they take the nuclear stain is responsible for the assertions that they have a nuclear origin.

Polychrome methylene blue. Here the epithelial cells are characterized, when viewed from the surface, by relatively thick unstained cell boundaries which mark the polyhedral cell boundaries. The unstained spherical nuclei occupy the central portion of the cells. These are surrounded by unstained cytoplasm with highly unstained refractile granules. The nuclei, however, begin to stain early in this dye, thus indicating the toxic effect of the stain.

The granules of the mast cells rapidly stain metachromatically and the nuclei, as in the case of neutral red, are unstained. Later they begin to stain, as in the case of the epithelial cells.

Janus green. After the tissue has been in this solution for a few moments the connective tissue elements are stained, under macroscopic observation, metachromatically a pinkish violet or purple, while the epithelial cells are stained blue. When the tissue is studied by means of the oil emersion, it is seen that the mitochondria of the epithelial cells are deeply stained blue, while the other elements of the cell are unstained. The mitochondria are numerous and show the same characteristics in both structure and distribution as observed in fixed preparations. A narrow, definite zone of homogeneous, unstained cytoplasm immediately surrounds the nucleus, in which no mitochondria are seen. This frequently gives to the nucleus a double contour appearance.

The mitochondria in the epithelial cells of the choroid plexus are indeed numerous. They are found in such great numbers that under low power the cytoplasm appears to be a solid blue throughout. It is only under the oil immersion that the minute

individual granules are observed. These granules vary in form. Some are spherical, others are slightly elongated, either slender or thick, others again are comma shaped. In no instance are long rods seen. It is the mitochondrial granules that are seen as the minute, highly refractive granules in fresh tissue, as well as in the various other vital stains used, where they remain unstained. In the janus green solution all the granular elements of the epithelial cells are stained blue. It is my opinion that the granules that have been described by previous investigators as secretion granules are in reality mitochondria.

The mast cells are unaffected in this dye. Only rarely does one see granules stained with janus green. Frequently one observes a fine, intergranular deposit of blue stain between the granules of the mast cells. I was unable, however, to make out definite mitochondria in this deposit.

Neutral red and Janus green. When both neutral red and janus green, mixed, are applied to fresh choroid tissue a very interesting picture results. The epithelial cells are deeply stained blue owing to the numerous mitochondria they possess. On the other hand, the mast cells are stained deeply red as a consequence of the affinity between the granules and neutral red. Careful examination of numerous mast cells did not reveal the presence of blue stained intergranular mitochondria. In fact, the complete absence of the latter was the striking and surprising feature, in view of the claims of Arnold and Dubreul that mitochondria are found in mast cells. Tochasehin ('12) reports staining of true mitochondrial elements in fibroblasts and clasmatoocytes by isamine blue and trypan blue. In this preparation, when enclosed within a warming stage, I observed processes projecting themselves outwards from cells in which granules concurrently appeared but no mitochondria were observed. This observation is against the view that some have held—namely, that the granules of mast cells have their origin from mitochondria. These observations confirm those already described for the fixed preparations.

Methylene blue. Two preparations of this stain were used. Methylene blue rect. Grüber's, and Methylene blue, Metz.

The former stained both the epithelial cells and the granules of the mast cells a deep blue. In the latter stain the granules of the mast cells are stained metachromatically a reddish violet. In neither preparation were nerve cells and fibers observed accompanying the blood vessels. However, claims have been made that numerous non-medullated nerves are seen in the vascular walls of the choroid plexus.

Pyronin. The epithelial cells are stained deeply red. The stain is diffuse and confined to the cytoplasm in which the highly refractile, unstained mitochondrial granules are observed. The mast cells are not stained. No diffuse stain was seen surrounding the mast cells. This observation was made with a view of determining if a secretion substance is present which had been emitted from mast cells as a consequence of solution of the granules. Arnold obtained a red stained substance surrounding mast cells after vital staining with weak solution of methylene blue. This he interprets as a secretion, thus ascribing to mast cells a glandular function. Others have claimed that the granules of mast cells, especially leucocytes, go into solution. I was not able to verify this observation of Arnold, either in the pyronin or methylene blue stains.

NATURE AND ORIGIN OF MAST CELLS

Many theories have been advanced respecting the histiogenesis of mast cells. Lymphocytes, mononuclear leucocytes, basophilic myelocytes and bone marrow (Schridde, '96) are generally cited as giving origin to mast-leucocytes. That mast-leucocytes have a common origin along with other polymorphonuclear leucocytes is probable.

No unanimity of opinion exists, however, regarding connective tissue mast cells ('histiogenen mast-zellen'). That they have origin from mast-leucocytes and consequently from the same sources as the latter is held by many observers. Prenant, Bouin and Maillard regard the two as belonging to the same category. Ranvier ('00) held that his elasmocytes were mast cells, that they had origin from blood cells and that they were associated with inflammatory changes. Schreiber and Neuman ('01)

agreed with Ranvier that clasmocytes and mast cells belong to the same type. Maximow states that the relation of the two—mast-leucocytes and histiogenen mastzellen—is not clear. Ehrlich held that the connective tissue mast cell is a transformed or supernourished connective tissue cell, that the granules of the latter are stored albuminous substance, and that the cells are found where tissue juices are present in abundance. Baumer ('96) agreed with this conception. That the cells held a reserve substance was also claimed by Schneider ('02). Ballowitz ('96) found them to be especially numerous in hibernating animals. Others again hold that these mast cells are related to the formation of fat, as they are frequently seen in adipose tissue.

Unna ('91) saw the transformation of certain spindle or ellipsoidal cells into mast cells in healing syphilitic ulcers. Mast cells are frequently found under pathological conditions, such as in low-grade inflammations, urticaria pigmentosa, erythema multiforme, pleuritic exudates, etc. Joachim ('06) and Spilling described mast cell leukemias.

Some have made the claim that mast cells are not normally present in human blood. Michaelis ('02), Wolff ('02) and many others have found them to be normally present. Audry ('96) claims that connective tissue cells, large mononuclear cells, wandering cells and plasma cells may become mast cells. That these cells may have their origin from blood cells and vascular anlage was held by Marchand ('97).

Recently much attention has been given to discussions of the origin of vascular endothelium and blood cells. That the former may develop directly from the mesenchyme is claimed by McClure ('16), Reagan, Clarke ('16), Danchakoff ('16) and others. Clarke ('12) on the other hand maintains that lymphatic endothelium is formed from preëxisting lymphatic endothelium and that the mesenchyme retains throughout its identity as a mesenchymal cell. That red blood cells develop directly from endothelium (even when discontinuous and independent of the circulation, Reagan) has been shown by the works of Reagan ('16), Danchakoff ('16), Emmill ('16) and Jordan ('16). These observations are more or less at variance with the Angioblast

theory of His. Stockard ('15), on the other hand, did not observe in the embryo of the Teleost, *Fundulus heteroclitus*, any indications "that an endothelial cell has the power to produce a blood cell or to change into a blood cell of any type but much has been seen to the contrary." According to him, the "four distinctly different products differentiating from the apparently similar wandering mesenchymal cells" occur under the same environmental condition. His explanation of this phenomenon is that the original mesenchymal cells that wandered out were of four potentially different classes. "The four resulting types of cells are then in an embryological sense derived from different mesenchymal anlagen," although these cannot be differentiated. The term polyphyletic theory has been applied to this conception. Against this theory is the so-called monophyletic theory which is ably defended by Danchakoff and Reagan. In substance it is this: There is one common anlage for all blood cells, the later differentiation into the various types of blood cells is determined by environmental conditions.

To review the arguments favoring each theory would be irrelevant in this discussion. Danchakoff, after extensive observations on both the embryo and adult chicken concludes that all blood cells including mast cells, plasma cells and wandering cells have origin from a common anlage,—mesenchymal cells—and that this loose mesenchyme of a chick embryo (6–10 days) is equivalent in all the regions of localization and is polyvalent in its potencies of development. Further, Danchakoff found in the lymphatic nodes scattered in the loose connective tissue of the adult hen a loose syncytium which is considered as young undifferentiated tissue. From these loose syncytial cells amoeboid cells develop and these are the stem cells, or mother cells, of the various formed blood elements. Regarding the rôle that the stem cells play in the production of various blood cells and wandering cells, Danchakoff ('16) states:

The erythrocytes, the small lymphocytes, the different leucocytes, the wandering cells of the connective tissue, the mast cells and the plasma cells—all these cells are different cell units, morphologically as well as physiologically. But in the early embryonic stages they all had

a common mother cell, and this mother cell is preserved in the adult organism and becomes the source of differentiation and regeneration and most probably also the source of pathological proliferation.

Many diverse opinions have been held regarding the nature of the granules of mast cells. Some have regarded them as albuminous in nature, while some regard them as mucinous. That they are associated with pathological changes in the cell and are products of inflammation or degeneration has been maintained by others. Stoffer held that they were related to the production of melanotic pigment. Pappenheim did not regard these granules as living,—‘biophoren Plasmosomen’—but a ‘substanz depot.’ He bases this view upon his observation that the granules can be extruded from the cell without losing their staining characteristics. Arnold, however, strongly maintains that mast cells are not to be regarded as cells in various stages of degeneration, but that they are active living normal cells. According to him, the presence of glycogen, fat, lipoids, pigment and mitochondria is sufficient evidence of this. Others hold that the mast cell granules are secretory in nature and consequently the cell is to be regarded as a unicellular gland. The chemical structure of the granules is unknown. According to many observers, they have their origin from the nucleus,—Weidenrich ('11), Downey ('13) and others. Arnold holds that they are related to mitochondria. Other substances found in mast cells, according to various investigators, are glycogen, fat, and pigments.

Many of the theories respecting the function of mast cells have been suggested in the two preceding paragraphs. In addition to these, Fahr has held that they exert a bactericidal or antitoxic action. That the cells are to be regarded as unicellular glands is an interesting conception. This view was held by Lavdowsky, Colleya and others, who claim that the granules become disintegrated, go into solution, and pass out of the cell to become mixed with the tissue juices, thereby contributing to the nourishment of the latter.

DISCUSSION AND CONCLUSIONS

The chief purpose of this paper is to call attention to the numerous interstitial granule cells in the choroid plexus of the ox, which possess the morphological and staining characteristics of mast cells of the 'histiogenous' or connective tissue type. Owing to the unavailableness of the ox, or the other mammals in which these cells were found in fewer numbers, for experimental purposes, there are many problems respecting these cells which remain unsolved for the present. One is almost constrained to believe that their origin is from the blood, for in sections they are seen first beneath the endothelial layer of the arterioles with processes extending into the lumina; then in the walls of the blood vessels at varying distances from the lumina, and finally beneath the epithelial layer of the choroid plexus with processes projecting between the epithelial cells into the ventricles. The entire picture suggests that the cells are traveling from the blood vessels to the ventricles. The fact that they are seen in such variable numbers in different choroid plexuses strongly suggests this view. However, I have repeatedly attempted to find these cells in both the blood and cerebro-spinal fluid without success. It was my custom to draw off with a glass tube and rubber bulb from the ventricles, cerebro-spinal fluid whenever the opportunity presented itself. About 5 cc. of fluid can be obtained in this way from the ventricles. Of course, there is some admixture of blood with this fluid, owing to the customary methods of slaughtering these animals. The fluid was then centrifugalized.

I have been unable to confirm that these granular cells regularly pass from blood vessels to the ventricles, although permanent preparations strongly suggest such an activity. Further, I have not observed mast cells passing from blood vessels in living tissue when mounted in serum.

My observations so far do not disprove the hypothesis of Ehrlich that they are highly or over-nourished connective tissue cells. It is feasible to assume that they are wandering cells, or a special type of connective tissue cell that have become granular as a consequence of their migration through the connective tissue

wall of the choroid plexus. That they may originate entirely within the connective tissue wall is suggested in view of the fact that they were seen to undergo cell division. Certainly there is a large amount of cell nourishing substance—cerebro-spinal fluid—passing along the course of these cells from the blood vessels to the ventricles.

Should we accept Ehrlich's conception, the mast cell cannot be regarded as a specific cell, but rather as a condition of a more or less general type of connective tissue cell. Against this theory, however, is the fact that the formation of granules occurs simultaneously with the development of processes and that in cell division the granules are abundantly seen in the two daughter cells during the process of division.

That they are not specifically related to fat formation in the choroid plexus seems certain, for fat is found in this tissue only in very minute quantities.

The cells are in no way related to any demonstrable pathological conditions. Hence the assumption that they represent abnormal processes is untenable. Likewise, they cannot be considered as related to the formation of pigment.

That the cells are concerned in some endocrinous secretion early suggested itself. Their presence in such constant and large numbers, their structure and staining characteristics, which in many respects resemble that of other gland cells, might indicate this. It was not until basic stains were used and the cells were seen to stain metachromatically with polychrome methylene blue that they were placed in the category of mast cells. Notwithstanding this fact, they may be concerned in some type of secretion. Many observers still regard mast cells as having a secretory function. Against this view of the secretory function of these cells may be advanced the fact that they are found only in a few mammals—in the ox, in great abundance, less numerous in the sheep, and only sparsely in swine. Further, no demonstrable physiological action was obtained after injection of ox choroid extract into the dog. I was unable to demonstrate a pericellular secretion substance, as observed by Arnold. Proof is still wanting that these cells are concerned in the secretion of some specific substance.

The apparent migration of these cells through the choroid plexus from the blood vessels to ventricles and the presence of inter- and sub-cellular canaliculi suggest that the cerebro-spinal fluid, at least in part, may follow such a course and consequently be independent, to some degree at least, of the surface epithelial cells.

The chief value of this paper is to point out that the choroid plexus of the ox offers excellent facilities for teaching and study of mast cells.

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INTESTINAL HERNIA IN TWO SPECIES OF FROGS

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THREE FIGURES

In considering the survival of the fittest we often find examples in man and other mammals of individuals who are somewhat handicapped by mental and physical defects but who are able to exist by their own labors. While intestinal hernia is not a great hindrance to normal activities in the case of civilized man, it is often a contributing cause to the death of some domesticated or wild mammal.

Recently two cases of hernia of the small intestine in the frog came to my notice and seemed worthy of mention in view of the paucity of recorded cases of such defects in vertebrates below mammals.

The first specimen, a young bullfrog (*Rana catesbiana*) was shown to me by a student in General Zoology, Mr. Charles Hruby. It was an apparently healthy and vigorous male with a body length of $3\frac{3}{4}$ inches. No anomaly was visible externally but the small intestine was curiously out of position. From within 4 cm. of the pylorus to within 3 cm. of the rectum the duodenum and ileum were entirely protruded through a small aperture in the abdominal wall to occupy a position in an enlarged lymph space just under the skin of the right side (figs. 1 and 2).¹ The pancreas was about 2 cm. long and lay in the mesentery between the pyloric end of the stomach and the duodenum. The duodenum extended about 2 cm. beyond the pancreas and became directed to the right side of the animal, finally passing through the muscular wall. Through the same opening, the ileum extended to join the rectum. Examination of the parts extruded showed them to be much coiled and closely

¹ Photograph by Mr. F. H. Dodge, drawings by Mr. Richard Ashman.

folded. The coiled portion was a little more than 8 cm. in length when straightened. The spleen was in its normal position in the abdominal cavity and the rectum and stomach were perfectly normal. There was no anomaly of the reproductive or



Fig. 1 Photograph of intestinal hernia in *Rana catesbiana*

excretory systems, and the blood supply of the whole intestine was complete.

The second specimen was presented to the writer by a former student, Mr. T. C. Nelson of the University of Wisconsin, who kindly offered to add it to my teratological collection.

It was an adult male leopard frog (*Rana pipiens*) measuring 3 inches body length and externally presenting no appearance of deformity. Careful examination of the organs other than the intestine showed them to be normal (fig. 3). The duodenum extended about 4 cm. from the stomach as in the larger specimen, then passed out through the body wall and continued as a single loop for 5 cm. before it recurved and passed through the same aperture into the body cavity and connected with the rectum at

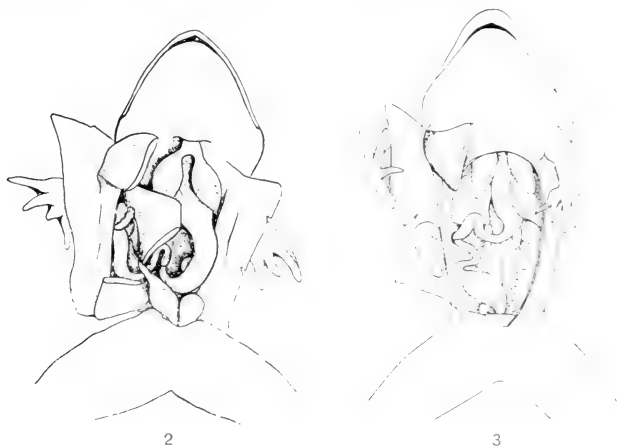


Fig. 2 Intestinal hernia in *Rana catesbiana*

Fig. 3 Intestinal hernia in *Rana pipiens*

a distance of about 3.5 cm. In both cases the cavity was soft walled and showed but slight thickenings of its walls due to abrading action of the intestine.

It is possible that early in the tadpole stage when the first elongation of the duodenal loop occurred, there was enough force exerted to break the body wall opposite the curved intestine; this seems quite plausible when we note that the extrusion took place at about the same point in the two specimens. Another less likely possibility is that mechanical injuries (on the right side

in each case) caused by crayfish, fish, frog, or turtle, produced sufficiently large apertures to allow the growing intestine to force its way out. In any case it is evident that frogs may live apparently unhampered by quite considerable hernias of the small intestine.

OBSERVATIONS ON THE INFLUENCE OF ISOLATED
OVARIES ON THE BODY GROWTH OF THE
ALBINO RAT (*MUS NORVEGICUS*
ALBINUS)

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TWO CHARTS

Previous experiments had shown (Stotsenburg '13) that the removal of both ovaries from a young rat was followed by an acceleration of body growth. When one ovary only was removed, the other being left untouched, the body growth was not modified (Stotsenburg '13) but the remaining ovary underwent a marked hypertrophy (Hatai '13).

The experiments to be reported were designed to test the question whether the exclusion of the reproductive function of the ovary, by isolating it from the uterus, would produce any general change in body growth.

Two series of experiments were made:—one series in which both ovaries were isolated and left in place—designated 'double isolation'—and a second, in which one ovary was isolated and left in place, while the other was completely removed—designated 'single isolation.'

TECHNIC

Each test animal underwent two operations—about ten days apart. In the case of the double isolation the procedure was as follows: About 1 cm. of the right uterine horn was removed at the first operation—and at the second, the same length of the left horn. In the single isolation the first operation was as given above—but the second consisted in the complete removal of the left ovary. All the operations were successful. The albino rats were thirty-two to thirty-five days old at the time

of the first operation and forty-five days old at the time of the second. At the date of the last operation and for the first forty-five days after the last operation, the body weights were taken at intervals of fifteen days, then at intervals of thirty days, until the termination of the experiment at two hundred days.

In the further description of the work the two series of experiments will be treated separately.

DOUBLE ISOLATION

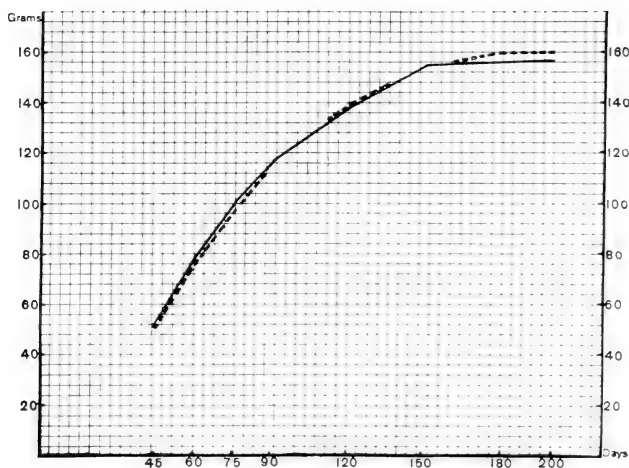
By the method just described, both ovaries were isolated in 20 females which were compared as to their body growth with 16 controls of like age. These 36 animals were from 7 litters, and as far as possible the same litter was made to furnish both control and test animals. The growth of the controls and of the test animals after double isolation is given in table 1 and shown in chart 1.

TABLE 1

Giving the average body weight of 20 test rats—'double isolation' and 16 controls, from which the graphs in chart 1 were plotted

AGE IN DAYS	TEST ANIMALS. NUMBER OF ANI- MALS	DOUBLE ISOLATION AVERAGE BODY WEIGHTS	CONTROLS. AVERAGE BODY WEIGHTS	CONTROLS. NUM- BER OF ANIMALS
		<i>grams</i>	<i>grams</i>	
45	20	49.4	51.1	16
60	20	76.5	79.2	16
75	20	98.5	101.1	16
90	20	116.0	116.7	16
120	20	140.0	138.0	16
150	20	154.0	150.0	16
180	20	160.0	156.0	16
200	20	160.4	156.8	16

From these data it is evident that this operation does not modify the growth of the body in weight. An inspection of the isolated ovaries at the time the test rats were killed yielded the following: There were 20 animals examined, in 7 rats both ovaries were not evidently diseased, but in 6 of these they were under weight and in one animal over weight. On the average these 14 ovaries were 13 per cent less in weight than was to be



expected from the tables for the normal ovaries (Donaldson '15). In 12 rats there were pathological changes. In 3 rats both ovaries were pathological. In 9 rats one ovary was pathological. In one case one ovary had disappeared. There were therefore in all 15 pathological ovaries to be considered. In the 12 rats with pathological ovaries—showing cysts—there were 15 cysts. These were distributed 11 on the left side and 4 on the right side. The first and earlier operation was on the right side.

SINGLE ISOLATION

For this series 17 test animals were subjected to the operation for 'single isolation' as previously described. There were 14 controls, and 7 litters were represented in the entire series. Test and control animals were taken as far as possible from the same litter. The growth in body weight of the rats after single isolation is given in table 2 and represented in chart 2.

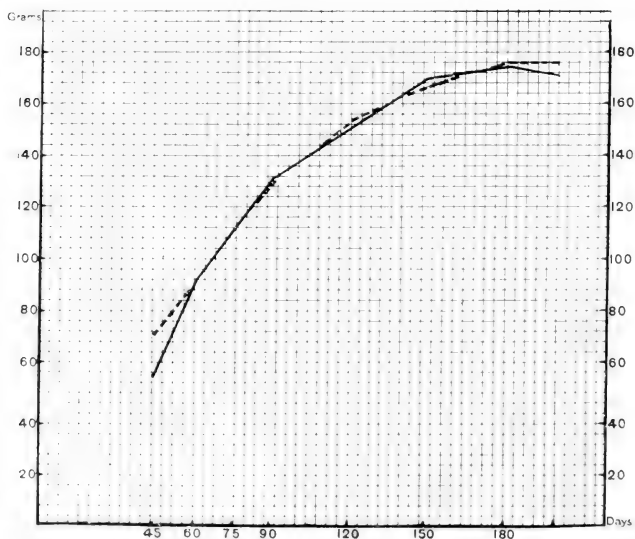
As shown by these data, isolation does not modify the body growth. An inspection of the isolated ovary at the time these rats were killed yielded the following:

TABLE 2

Giving the average body weights of 17 test rats—'single isolation'—and 14 controls, from which the graphs in chart 2 were plotted. Several animals in the test series were removed at one hundred and eighty days because of lung infection, and several after one hundred and fifty days from the control series for the same reason. Test animals—single isolation.

AGE IN DAYS	NUMBER OF ANIMALS	TEST ANIMALS. AVERAGE BODY WEIGHTS	CONTROLS. AVERAGE BODY WEIGHTS	CONTROLS. NUM- BER OF ANIMALS
		grams	grams	
45	17	69.6	56.9	14
60	17	90.0	90.7	14
75	17	112.3	113.9	14
90	17	130.3	131.4	14
120	17	144.6	150.9	14
150	17	166.9	171.0	13
180	14 ¹	175.6	174.0	12
200	15	175.3	171.1	11

¹ Weighing of one rat accidentally omitted.



There were 15 test animals examined. In 10 the ovary was not diseased. In 5 the ovaries were pathological, being represented by cysts. The weights of the 10 normal ovaries were as follows: one was 44 per cent less than the table value, the remaining 9 were all above the table values. On the average these 9 remaining ovaries weighed 148 per cent more than normal. They were therefore considerably over twice the normal weight to be expected from the reference tables (Donaldson, '15). It is to be noted that in the reference tables the weights are given for both ovaries taken together.

CONCLUSIONS

1. Both the double and single isolation experiments show that the glandular function of the ovary which affects body growth is unmodified when the ovary is isolated from its connection with the uterus—since the isolated, acts like the normal ovary to inhibit growth.

2. This inhibition of growth is exercised in the case of 'double isolation' by ovaries that appear under-size or pathological at the end of the experiment (200 days), while in the case of the single isolation experiment—ovaries, for the most part greatly hypertrophied, exercise the same control.

3. From the fact that the same effect follows from ovaries in such different conditions it would appear that they probably exercise their control through the mediation of some other less modified member of the endocrine system.

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ANTERIOR HAEMATOPOESIS IN CHEMICALLY TREATED TELEOST EMBRYOS UNDER CONTINUAL OBSERVATION¹

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ELEVEN FIGURES

Inhibition of the movement of body-fluids has recently been employed for the purpose of obtaining information concerning the normal origin of teleost blood corpuscles. Those investigators who have employed this method of study have emphasized the importance of a thorough knowledge of the history of the experimental material from which conclusions are to be drawn. For instance, it has been stated (10, p. 579) that if an investigator knows the entire history of a given embryo, having determined during that time the embryo in question has never had any circulation whatsoever, he will invariably find that blood cells are located only in the intermediate cell-mass and on the posterior and ventral yolk surfaces; that there may be some variation but none "sufficient in any case to confuse the problem;" that erythrocytes in such embryos (8) will never be found in the anterior mesenchyme, anterior vessels, anterior yolk surface, heart or liver, "in any embryo at any age;" that in these latter regions (9, p. 315) wandering blood anlagen never make themselves manifest. Along with these statements, no claim is made that their author has followed in sufficient detail the history of the development of any embryo to justify an assertion that the complete history of any embryo was known. Perhaps the most

¹ The present communication represents a portion of a work involving continual observation which was aided by a grant from the National Academy of Sciences.

definite available statement in this connection (10, p. 578) is the following:

"Since one is able to be *absolutely certain* that the blood never circulates in a great number of embryos, only such embryos should be considered in a study of blood origin."

It is evident that if one should use as his criterion for lack of circulation a corresponding lack of erythrocytes in certain locations—if he should refuse seriously to consider all embryos with erythrocytes in these locations because of pictures which can be obtained in embryos by the arrest of a once established circulation, he is using a method capable of giving only one result.

On the other hand, it has been suggested (5, p. 104) that this same thoroughness of knowledge of a given embryo's history, such as could be obtained only by constant observation, might conceivably heighten the probability of a detection of erythrocytes in those locations in which they have been claimed never to originate.

It has been stated that circulation, after having once been established, may be lost temporarily intermittently or permanently. Temporary or intermittent loss of circulation would, of course, lead to error of interpretation, provided that circulation were of a sufficiently elusive nature to take place only between those intervals at which a single investigator could reasonably be expected to make his observations. It seems that the only means of meeting this difficulty of elusive circulation (if there be such a phenomenon) is to examine as often as possible the experimental material. Since the working hours of a single investigator might be inadequate for the elimination of the objections which might be raised by believers in elusive or intermittent circulation, the problem would best be attacked by the coöperation of a sufficient number of observers that the probability of detection of an elusive circulation approaches a maximum. It is, however, impracticable to observe with absolute constancy any single embryo. In the first place, it is impossible to predict which chemically treated embryos of a large number will succumb to treatment, develop a circulation, or develop without a circulation. Since embryos of the latter sort are generally few

in number compared with the other two sorts, constant watching of only one individual would necessarily involve a waste of time, and would no doubt be superfluous, even if one could be sure that a given embryo would develop without a circulation. At any rate, the method in the present work was continually to eliminate from a group of chemically treated embryos, those individuals which developed a circulation, and those which died or became extremely abnormal. Such groups of treated embryos were carefully observed day and night at intervals varying from thirty minutes to an hour or slightly more. Low and high power binocular microscopes were employed in these observations. In order that individual cases might receive adequate attention, it was necessary to limit the number of embryos very considerably below that which might be studied if observations were to be made only a few times each day.

The primary purpose of the present work was to obtain a definite answer to the simple question: are erythrocytes ever found in the anterior mesenchyme or in any anterior vessels of teleost embryos in which the blood has never circulated? A negative answer to this question has been insisted upon by Stockard, while a positive answer has been likewise insisted upon by Reagan. The possibility of migration of erythrocytes or their anlagen is entirely beside the question. The issue concerns the presence or absence of erythrocytes in this location regardless of all possible means of attaining that location with the exception of passive movements induced by heart pulsation.

Reagan (5, p. 116) has pointed out the fact that Stockard (8) has described erythrocytes as 'originating' on the yolk when their ultimate anlagen must have come from another location; the former has also pointed out the fact that even the leucocytes and their anlagen, as described by Stockard, were unable to migrate. In consideration of ultimate origins it is well to remember that the intermediate cell-mass itself arises by the gross mesial displacement and subsequent union of two longitudinal columns of mesenchyme.

It may be stated at once that a small number of embryos which developed without circulation, as was determined by fre-

quent observation, possessed erythrocytes in their anterior mesenchyme. It has previously been admitted (5, p. 112) that a large number of embryos without circulation may fail to exhibit red blood cells in their anterior regions. It was first pointed out by Reagan that blood shares with other tissues the especial susceptibility of the anterior end of the embryo to a derangement of metabolism. One can readily obtain large numbers of embryos devoid both of circulation and of anterior erythrocytes; but if the middle and posterior regions of such embryos were found to be as greatly arrested and abnormal as the anterior portions generally are, they would be rejected as material in which to study the origin of the vascular tissue or the origin of any other tissue. This specific behavior of the anterior tissue is included among the extensive studies by Child on Axial Gradients. To confirm the work of Stockard, one must reject all embryos exhibiting anterior erythrocytes regardless of their histories, and maintain that in the remaining embryos with diminutive anterior ends, erythrocytes have developed in all those places in which they could ever develop in the normal individual; one must claim that there is no possibility of varying degrees of haematopoietic potentiality. It seems unreasonable to assume that all regions should exhibit equally strong tendencies to produce blood cells, or that they should all necessarily produce them at the same early period of ontogeny. Further study alone will determine the reasonableness of this assumption.

The material for the present work consisted of a few hundred embryos of *Fundulus heteroclitus*, which, at a very early stage, were treated with chemicals. The conditions in one of several satisfactory embryos obtained (i.e., having anterior erythrocytes) will suffice for descriptive purposes. The known history of this embryo will be given in detail. The eggs constituting the group from which this embryo was taken were fertilized at 4 p.m., June 22, 1916. At the two cell stage, they were placed in a solution consisting of 70 cc. of sea water to which had been added 10 cc. M/12 butyric acid. Four hours later the solution was diluted by the addition of 50 cc. sea water. In this mixture they remained until the total time of treatment amounted

to twenty-four hours, after which they were reared in sea-water which was frequently changed. From the time of their removal from the solution of butyric acid the eggs were frequently observed. Recorded observations were commenced on June 26 at 8 a.m., although at this time there was no sign of endothelium on the yolk-sac. Examinations were made as follows:

June 26, a.m., 9.20, 10.30, 11.45; p.m., 12.20, 1.30, 3.30, 4.45, 5.34, 6.11, 7.00, 8.05, 9.02, 10.00, 10.55, 11.54. June 27, a.m., 1.00, 2.32, 4.00, 5.15, 6.30, 7.50, 9.00, 10.15, 11.20; p.m., 12.10, 1.30, 2.11, 3.26, 4.19, 5.30, 6.14, 7.35, 9.00, 10.55. June 28, a.m., 12.30, 2.00. (So far there had developed no pigment on the yolks and none of the embryos possessed pericardia. Constant observation up to this time was quite superfluous.) 3.12, 4.15, 4.45, 6.00, 7.00, 8.02, 9.14, 10.25, p.m., 1.45, 3.14, 4.15, 5.11, 6.30, 8.02, 9.00, 9.55, 11.00. June 30, a.m., 12.20. At this time the embryo from which figures 1 to 11 are taken was found to contain anterior erythrocytes; this embryo was thoroughly studied, sketched and then preserved in picro-acetic acid. It might be of interest to note that none of the (approximately) one hundred individuals obtained in this same treatment had yet established a circulation. A little later, one of those embryos was found in which heart-pulsation was able to cause oscillation of the blood-corpuscles. This embryo was discarded; then twelve hours elapsed before another such embryo was found, observations having been kept as constantly as in the earlier stages.

One embryo in which anterior erythrocytes (figs. 1, 9 and 11) were observed, and the history of which has been given, is of great interest. The yolk-sac was observed just before the preservation of the embryo to be quite devoid of endothelium except in the most posterior region. This endothelium was readily found in section. Numerous pigment cells had developed on the yolk sac. Also there were many oil globules. On the posterior yolk were some large groups of developing blood cells which had yet shown little indication of possessing hemoglobin. They were detected by means of a high power binocular. Their color was only a little different from that of the surrounding mesenchyme, but their shape and refracting power were characteristic. In the posterior region of the intermediate cell-mass the developing erythrocytes had assumed a faint yellow color. There was not the slightest evidence that any of these cells had been, or were being, displaced by any sort of movement of body-fluids. No

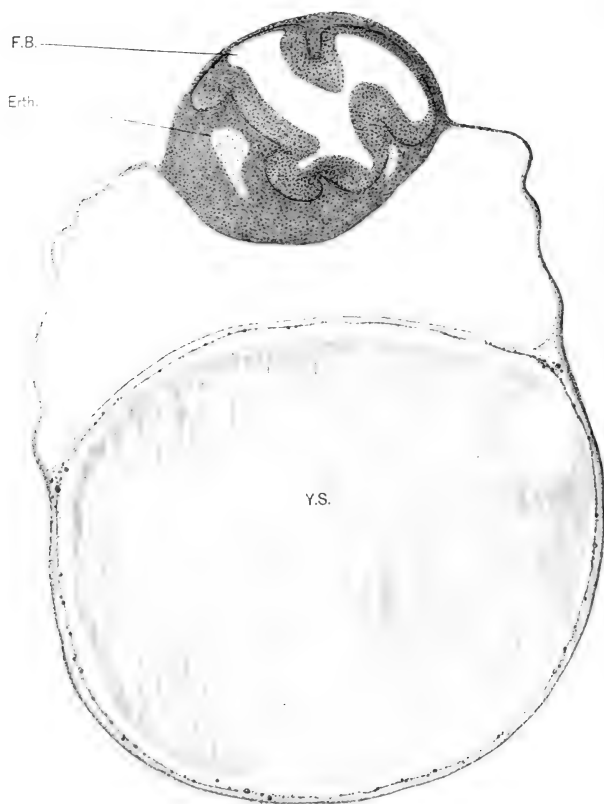


Fig. 1. Section through the anterior body axis and yolk sac of a chemically treated embryo from which all other figures in the present account were taken. The section shows lacunae of erythrocytes in the anterior mesenchyme. The yolk-sac is seen to be quite free from vascular tissues. Normally such a section should contain a cross-section of the heart. In this embryo the venous attachment of the heart is dorso posterior in the pericardial cavity. Continual observation exp. 2.53, 1916. *Erth.*, erythrocytes; *F. B.*, fore-brain; *Y. S.*, yolk-sac.

body-movements could be elicited from this embryo. The heart was very inconspicuous (fig. 8) and was detected with great difficulty. A very slight twitching could be observed on the ventral side of the head which gave much the appearance of slight irregular contraction of a body-muscle. The wave of contraction was directed anteriorly, appearing superficially to be quite opposite in direction from that generally found in embryos at this stage. This circumstance finds its explanation in the fact that the venous attachment of the heart to the yolk-sac is situated at the extreme dorso-posterior limit of the pericardium ventral to the axial body-tissue (figs. 8 and 10); in the normal individual the venous portion of the heart is attached to the yolk at the antero-ventral limit of the pericardial cavity. In this specimen there is no endothelial tissue near the venous end of the heart. Also there are no blood corpuscles near it, though two cells were found among the myocardial cells which had become faintly eosinophilous, and one was much rounded. The endocardium is represented by a very delicate cord of cells which are not sufficiently numerous to enclose a cavity (fig. 6). The myocardium is weakly developed. Considering the fact that this embryo is less than six days old, it seems improbable that any yolk sac endothelium should have formed and then degenerated. That endothelium which could be observed in the living condition was readily found in section. At any rate, continual observation established the fact that there was no circulation. In the living condition the body tissue was clear and of healthy appearance. It seems quite likely that the prevention of circulation in this instance may have been due to an inherent abnormality of the heart. The anterior end of this embryo was so little affected, judging at least from external appearances, that a circulation might well have developed later, provided the heart had attained its normal relationships. It is impossible to state to what extent the chemical treatment is responsible for this heart abnormality.

Embryos of this sort are very desirable for study. In many instances a local and apparently insignificant abnormality may be sufficient to prevent circulation in embryos whose general con-

ditions are such that a circulation might well be expected. Such embryos are certainly as useful, as far as the lack of circulation is concerned, as those in which the agent of arrest of circulation has produced generalized abnormality with specific injury to the anterior tissues. It is in the former type of embryos that anterior erythrocytes are most often encountered.

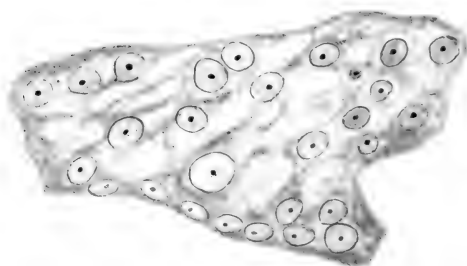
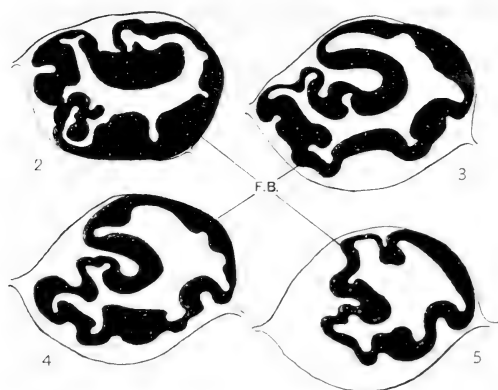
In figure 1 it will be seen that blood cells have developed in the anterior mesenchyme of the optic region, and the median dorsal fore-brain region. As has been stated, these cells possessed more hemoglobin than did the more posterior ones, though the former might easily have been overlooked in the living embryo.

The fore-brain tissue in this embryo appeared in the living condition to fill an abnormally large part of the head-region. The head-region itself seemed to be perfectly normal in size, differing in this character from the great majority of embryos in which circulation was prevented. In the fore-brain region, rather unusual developmental procedure is in evidence. There are several evaginations from the brain which bear a resemblance to optic cups. On each side there is a large cup and some smaller ones. Both dorsally and ventrally the fore-brain has given off evaginations which in some cases are so fused with the body-wall ectoderm (ectoderm in case of the dorsal and lateral ones) that no line of demarcation can be detected between the two ectodermal tissues (fig. 2). This hyperactivity of the fore-brain tissue is sufficiently often accompanied by the excessive development of anterior erythrocytes to warrant the suggestion that the two phenomena may be causally connected. Reagan (5, p. 107) has described a case in which supernumerary prosencephalic evaginations were accompanied by anterior erythrocytes.

Figs. 2 to 5 Sections through the fore-brain, showing hyperactivity of that tissue (encephalocoels). Exp. 2.53, 1916.

Figs. 6 and 7 High-power drawings of sections through the heart of this same embryo. Figure 6 lies in the arterial region. The endocardium consists of a single strand of cells. *Endc.*, endocardium; *Myc.*, myocardium.

Fig. 7 Section through the venous end of the heart in which neither myocardium or endocardium is distinguishable from mesenchyme.



In this connection the work of Child (2) is of extreme interest. He has found that long and severe treatment eliminates or prevents the formation of median or axial tissues, thus causing bilateral structures to fuse; that this fusion is most pronounced in the anterior-most tissues and progresses gradually backwards; that the originally distinct eye-spots of Planarians can thus be caused to fuse into a single median one. Werber (13) suggested vertebrate cyclopia to be an expression of this same sort of procedure. As a matter of fact the vertebrate otocysts themselves may be caused to fuse into a single median one which surrounds itself with a single otic capsule. As yet it had occurred to no one to suggest that the ears arise from a median anlage, as has been maintained for the eyes. But it has also been found by Child in a work which is not yet published, that while the anterior end suffers this specific injury with some treatments, other methods of treatment less severe and of slighter duration preceding removal to the normal environment will produce conditions under which the anterior end recovers with greater rapidity than does the posterior end, and a condition of hyperactivity may exist. Child was able by these latter methods to produce four-eyed Planarians. These results on Planarians are paralleled recently by the unpublished work of Child on Echinoderm plutei. By long and severe treatment, it was found that the morphologically anterior skeletal elements may be made to fuse, while in the case of weaker and shorter treatments, the angle of divergence between them may be made to approach 180° , while other anterior tissues become correspondingly hyperactive. While the recorded instances of anterior hyperactivity are few in the case of vertebrate embryos under treatment, it is possible that methods might be obtained whereby such hyperactivity might be obtained more often than by methods at present known to us. Quite independently of the results of Child, we have found as he has found in his unpublished work, that hyperactivity of the anterior end is often obtained when embryos are exposed to strong solutions for a very short time, followed by weaker solutions before removal to the normal environment. From such treatments, however, embryos may be obtained which appear

to have suffered neither arrest nor over stimulation in the anterior tissues. While such embryos may exhibit many anterior erythrocytes, the lacunae observed there are not so large as in those embryos whose anterior ends have been overstimulated.

There is one point of great importance in regard to the staining properties of early erythrocytes. When these are stained with methyl blue and eosin, or when they are stained with Wright's blood stain, the staining reaction is practically the opposite of that of older erythrocytes. Also the staining reaction of early erythrocytes in a single embryo may be opposite or very different in different regions.

In blood-smears from early embryos, such as four-day embryos, which seem to have been most successfully stained, the nuclei of the blood cells (all of which appear to be erythrocytes) stain reddish purple or red, while the cytoplasm appears blue or even densely blue and faintly granular; this is the picture obtained from Wright's blood stain. In eosin-methyl blue, the nucleus stains red or purple, while the cytoplasm appears dense blue, pale blue, greenish blue or in some cases very slightly purple. These staining-reactions of course may vary with the technique, but the general results above described are most often obtained.

In sixteen-day embryos, or even in stages younger than this, when the red corpuscles have well developed haemoglobin, the staining reaction is practically opposite that of erythrocytes of four-day embryos. In these advanced stages the best-stained preparations show erythrocytes with deep blue or purple nuclei and with deep red, pink or almost clear cytoplasm. If one employs such stains as the haematoxylin, the nuclei of all stages can be made to stain darkly so that even with a counterstain, the method seems incapable of demonstrating the nature of this remarkable change which takes place.²

² A reversal of staining reaction of nucleus and cytoplasm was observed many years ago by Auerbach. Wallin (*Anat. Rec.*, vol. 9, no. 6) observed a reversal of staining reaction in mesenchyme cells and in free blood-cells in the *anterior* mesenchyme of the larva of *Petromyzon*. Also this result was obtained from ordinary stains. No doubt the entire chromatin content of a cell

A most interesting case of variation in the staining reactions of erythrocytes within a single embryo is that exhibited by the individual which is figured in the present communication. As has already been noted, the anterior erythrocytes were more deeply red in the living condition than were those of the posterior region, the latter appearing only faintly yellow. It was possible to mount enough of the sections on one slide so that the staining conditions may be assumed to be sufficiently uniform in different regions to warrant the belief that actual differences are portrayed. The nuclei of the anterior erythrocytes stained homogeneously red and purple while the cytoplasm stained pink. The erythrocytes in the posterior part of the intermediate cell-mass were found to possess red and purple nuclei of granular character and pale blue or densely granular blue cytoplasm. The intervening regions exhibited transitional conditions between these extremes.

These peculiarities of the staining reactions of erythrocytes are worthy of consideration in connection with the anterior 'leucoocytes' described by Stockard (9, pp. 280 and 281). It is interesting to note that these 'leucoocytes' were found almost entirely in embryos about seventy-two hours old. It may be regarded as highly significant that their first recorded counterpart in the normal (?) series is found in a sixteen-day embryo, although the statement is made (9, p. 236) that the entire experimental series was checked by corresponding normal stages. The mere fact that these particular cells "stained differently from any other cells within the embryo" or that they are 'peculiar' (9, p. 280) certainly comprises insufficient grounds for their interpretation as leucoocytes. This hiatus in the production of leucoocytes between the ages of three and sixteen days is most puzzling. If it be true, as suggested by Reagan (5, p. 113)

may become oxychromatic. It may, however, be doubted whether Wallin is really dealing with acidophylic chromosomes. He shows no distinct chromosomes. It is possible that in his figures 5 and 6 he has cut diagonally through a spindle so that at one pole the section passes through a sheath of oxychromatic substance while at the other it passes through the central core of basichromatic. The latter, however, should in all cases show at least a thin periphery of oxychromatin.

that these are really abortive attempts at erythrocyte formation, and that these cells have, or have had the potentiality of elaborating hemoglobin, it seems difficult to explain the fact that they were never detected as erythrocytes in stages slightly later than seventy-two hours unless these were then discarded on the assumption that the blood had circulated. Stockard (10, p. 578) regards all the 'beautiful blood islands' and 'great clots' in the anterior end of the embryo as mere 'pitfalls' to correct observation. But if embryos containing anterior lacunae of erythrocytes must necessarily have had a circulation, it should still be possible to find these 'leucocytes' somewhere in the embryo. Diligent search for such leucocytes in embryos of this sort has many times proved fruitless.

A careful study was made of some of these embryos which were able to develop circulation following the chemical treatment; this involved frequent observation of about twelve hundred embryos. In every instance in which a circulation was known to have existed and then to have stopped, the embryo failed to re-establish a circulation and soon died. Two hundred embryos, furthermore, which had established circulation subsequent to chemical treatment were placed in a solution of 0.01 per cent potassium cyanide to which was added a rather strong solution of acetone. Examined after having been subjected to this severe treatment for one and three-quarters, five and one half, eighteen and one half, and twenty-eight hours, the embryos were found without exception to have retained their circulation. At the end of forty-eight hours only two of the two hundred embryos had lost circulation; these quickly disintegrated without having resumed circulation.

From the foregoing results, the conclusion seems justified that the intermediate cell-mass and the posterior and ventral yolk surfaces are not the only locations in the teleost embryo which are capable of giving rise to red blood cells. Just to what extent any experimental condition represents the normal is difficult to say. The fact that a great many embryos with arrested development fail to show anterior erythrocytes might conceivably militate against the belief that the anterior mesenchyme normally

contains erythrocyte anlagen. It is, however, a very significant fact that the more normal the general conditions to be found in such embryos, the more likely they are to develop anterior erythrocytes. In the greater number of cases of developmental arrest, those conditions which will prevent circulation will also prevent the formation of anterior erythrocytes.

It seems probable that when an embryo is once able to establish a circulation, it has little tendency to lose that circulation under favorable environmental conditions. When loss of circulation does occur, the embryo usually dies. We have found no case of temporary loss and re-establishment of circulation—much less, any sort of elusive or intermittent circulation. Also there has been no instance in which a pericardium was observed to become oedematous or the heart string-like, once the circulation had been established. We do not assert that such things never happen. As a matter of fact, one does well to refrain from sweeping generalizations of a negative character in discourses dealing with the developmental possibilities of chemically treated embryos.

It is not our purpose to apply the facts above presented to considerations of mono- and polyphyleticism, except to state that they furnish a positive disproof of one of the contentions upon which support of the polyphyletic view has recently been based. There are certain facts which can be determined; beyond this, all is mere speculation. One can determine three things concerning the origin of a tissue: first, whether it arises from a narrowly limited anlage; second, whether any other tissue can ever give rise to the tissue in question; third, whether the assumed anlage never gives rise to any other tissue except the one for which it has been claimed to be specific. If the development of a given tissue fails to conform to any of these three possibilities, polyphyleticism can never be proved.

There seems to be no little confusion as to the proper application of the results of the study of vascular tissues in the teleost to the Angioblast theory. Stockard believes that the studies on yolk-sac endothelium by Wenekebach, Raffaele, and himself, help to disprove the Angioblast theory.

Sabin (*Science*, August 4, 1916, p. 155) states: "In his (Stockard's) studies made on the yolk-sac of the living fish embryo, he found that endothelium arises as spindle-shaped cells which differentiate out of the mesenchyme. Moreover, he found that the endothelial cell was distinct from the blood cell. This confirmation of the angioblast of His I regard as very important."

The truth is that the observation of local formation of endothelium on the fish yolk-sac or on any other yolk-sac proves or disproves neither of the conflicting views. Both views admit of locally formed yolk-sac endothelium. The fact that the endothelial and corpuscular end-products of differentiation are distinct lends no more support to the Angioblast theory than to the Local Origin theory. If they were not distinct, how could we diagnose them? The statement that two differentiated products are different is simultaneously so correct that it is redundant and so redundant that it is correct. Their distinctness does not preclude community of descent; this is a fact which we grasp with great difficulty. The real problem is whether we will recognize or refuse to recognize the transitional stages between the end-products, provided such can be found. Until recently, most of us have refused to recognize the transitional stages which undoubtedly exist between mesenchyme and endothelium, and now that we have done so, there is a movement to attach to individual cells in this transitional stage the appellation 'Angioblast,' so that whereas there was originally one angioblast there are now as many thousands of them as the case may require. It is true that certain words in the sciences have undergone evolution in meaning; this is true of the word 'cell,' but this evolution did not serve to confuse the issues of a controversy. Inasmuch as Hooke did not consider a cell to be a vacuum, the term is not so inappropriate as it is sometimes considered.

Stockard objected vigorously to the account of the development of the blood in Keibel and Mall's *Human Embryology* for the reason that here the monophyletic view is (3, p. 350) accepted 'with open arms' (9, p. 310). But this account is written in strict accord with the Angioblast theory. It is difficult to reconcile polyphyleticism and angioblast, if angioblast be a 'unit

anlage' (3, p. 499) of which all vascular tissues are 'direct descendants'.

There have appeared recent discussions over blood-histogenesis in which authors would seem to believe themselves to have settled by words the entire question of preformation—a feat which Bonnet, about two centuries ago proclaimed as “une des plus belles victoires que l'entendement put ait remporte sur les sens.”

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PLATES

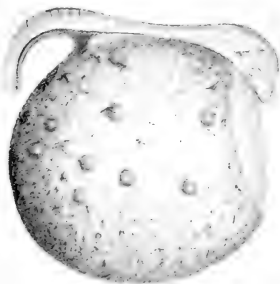
PLATE 1

EXPLANATION OF FIGURES .

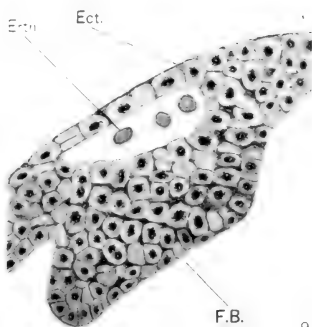
8 Sketch of the living embryo of which all other figures in this account represent sections or portions thereof. The erythrocytes in the head, tail and on the posterior yolk are represented by dots. The entire yolk sac except the posterior surface is devoid of blood and endothelium. There are large oil-globules on the yolk. Exp. 2.53, 1916.

9 Portion of a section through the fore-brain region in which the hyper-active brain-wall has fused with the ectoderm, enclosing on all sides some mesenchyme which became haematopoietic. There is no endothelium present. If any has been present it has either disappeared or turned into erythrocytes. *F. B.*, fore-brain; *Ect.*, ectoderm; *Erth.*, erythrocytes.

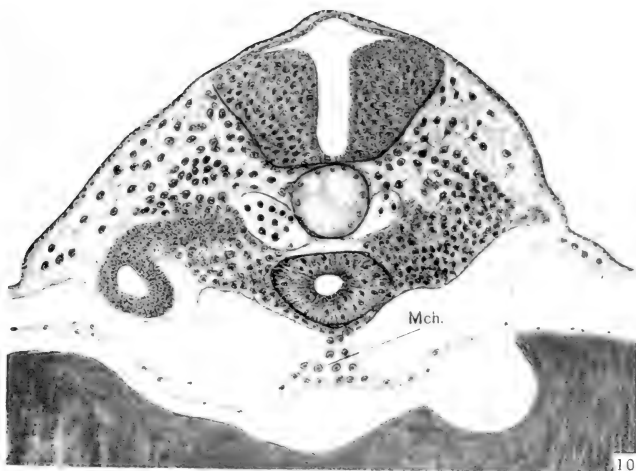
10 Section through the body-axis of the same embryo showing the loose mesenchyme (*mch*) in which the venous end of the heart loses itself.



8



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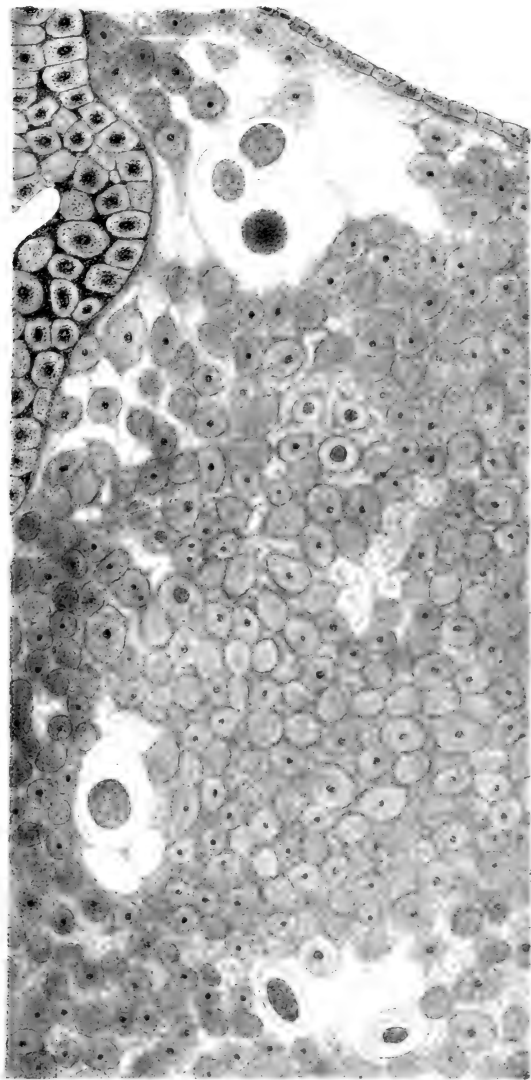


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PLATE 2

EXPLANATION OF FIGURES

11 Portion of a section through the fore-brain region of the embryo from which the previous figures were made. It was drawn at high magnification. Lying free in the mesenchyme are erythrocytes apparently in various stages of development. The epithelium with large cells and dark intercellular substance is prosencephalic tissue.



OESTRUS AND OVULATION IN SWINE

GEORGE W. CORNER AND A. E. AMSBAUGH

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I. THE PERIOD OF OVULATION

It seems remarkable that almost nothing should be known of the process of ovulation or of the mature ovum of the pig, which has for many years been in constant use for the investigation and teaching of mammalian embryology. In so thorough a review as Marshall's 'Physiology of Reproduction' ('10) there is only a meagre note:

It is probable that the sow ovulates during oestrus and not during the pro-oestrus, since it is stated that sows are most successfully served on the second or third day of 'heat.' Coition, if it occurs earlier, is frequently not followed by conception. From Hausmann's description (1840) it would seem that ovulation does not take place prior to coition, but this statement has not been confirmed.

That ovulation occurs at or near the time of oestrus is stated in Keibel's *Normentafeln* ('97) and is of course implied in Assheton's studies on the early development of the pig ('98), but the precise relation of the two events has never been fully worked out.

To obtain exact information upon this point we have undertaken the observations here reported. They have been made possible through the generous cooperation of Mr. J. O. Snyder, of the Western Meat Company, San Francisco,¹ and of Mr. Ralston B. Brown, Superintendent of the Oakland Meat and Packing Company's plant in West Berkeley, and his staff. Through Mr. Brown's kindness permanent laboratory space had

¹ We hope to have an opportunity to express fuller thanks to Mr. Snyder and the gentlemen of the U. S. Inspection service at the South San Francisco plant, in connection with other studies to appear somewhat later, the material for which has been obtained through their immediate assistance.

already been provided at the packing house; for this work we were allowed free access to the stockyards at all hours, and upon occasion animals were killed out of the regular order for us.

The females of the wild swine of Europe are monoestrous, according to Kaeppli ('08), having but one period of heat in the year; but under domestication the sow becomes polyoestrous, coming in heat at intervals of two to four weeks, usually about every twenty-one days, as all breeders agree. The period of heat commonly lasts three days and is characterized by sexual excitement and in some individuals by swelling, reddening, and slight eversion of the vulva, or even at times by a serous, mucous, or partially sanguineous discharge from the genital orifice. If a boar be present, the sexual excitement is made apparent by ready acceptance of coitus, which is denied at all other times; if none but females are in the pen, the sow in heat will be seen to sniff at the genitals of her neighbors and 'ride' them in imitation of coitus. Frequently the sow is the recipient, rather than the donor, of these attentions. The period is not terminated by coitus, but continues until the end of three days.

In order to distinguish these sows from others in the corrals until the time of butchering, they were marked with a daub of white paint thoroughly rubbed into the hair of the back between the shoulders. Immediately after evisceration, the Fallopian tubes were removed by cutting across the upper portion of the uterine horns, were carried to the laboratory in 0.7 per cent saline solution, and there washed out by inflating them with salt solution through a slit in the wall near the fimbriated extremity. After inflation with the fluid, the tubes were gently 'milked' into a Syracuse dish, and the washings examined with the dissecting microscope. This simple and almost infallible method of finding the ova was suggested to us by Professor Evans as an improvement on Martin Barry's practice of milking the tube without injected fluid ('39). As we have subsequently found it had been used by Sobotta and no doubt others as well.

Our series includes ten animals which were in heat on the day of killing or the day before. In eight of these the Graafian

follicles had ruptured and we were able to recover some or all of the ova from the tubes in each case. Of these eight sows, six were killed on the second or third day of the period, one between sixteen and thirty-nine hours after the onset of heat, and one between thirteen and twenty-two hours after the beginning of oestrus. In two of the ten there were large Graafian follicles, all unruptured except one follicle in one of the sows, which had apparently just collapsed. Unfortunately we have no record as to the time of onset of heat in these two animals, but the conditions in the other eight show that ovulation had occurred during oestrus, and probably on the first or second day of the period.

II. OVULATION SPONTANEOUS IN THE SOW

During the discussion which arose over Born's and Fraenkel's suggestion that the corpus luteum exercises the function of inducing ovulation at regular periods, a distinction was drawn between those mammals in which ovulation is spontaneous, and those in which copulation is necessary to invoke rupture of the follicles. Villemin ('08) maintained that ovulation is spontaneous in all mammals, but Ancel and Bouin ('09) state, on the basis of personal researches (details of which are not given) that ovulation is spontaneous in the human species and in other primates, in the dog, horse, cow, and pig; in the rabbit, guinea-pig, mouse, and cat rupture ensues only after coitus. The work of Marshall and Jolly ('06) on the dog and Heape ('97) on the mare, are in agreement with the results of Ancel and Bouin, and to the first mentioned class we may also add the sheep (Marshall '03) and the rat, according to Sobotta and Burekhard ('10) confirmed and extended by the recent carefully gathered data of Long and Quisno ('16). The placing of the rabbit in the second class has been confirmed by Regaud and Dubreuil ('08); the cat by Longley ('11); and Marshall ('04) has added the ferret to the list. The mouse, however, belongs to the class in which ovulation is spontaneous (Tafari '89, Sobotta '95) and also the guinea-pig (Loeb '11).

The only mention of the sow in this regard is the statement of Hausmann ('40) quoted above from Marshall ('10) that in this species ovulation is not spontaneous. On the contrary, our specimens show clearly that in swine coitus is not necessary for rupture of the Graafian follicles, for we have records of ten sows in which ova were found in the tubes although no boars had been in the pens with them. Mr. Brown informs us that according to the conditions of shipment of the live-stock, it is very unlikely that these animals had the opportunity to copulate before arriving at the stockyards. Two of the sows were under observation before oestrus set in, and are therefore even more definitely known not to have copulated. Moreover, in an animal in which but one follicle of many had ruptured, copulation had been observed sixteen hours previously. Ovulation, therefore, is independent of copulation.

III. THE MATURE OVUM

Little or nothing has been known of the mature ovum of the sow, and we have found no record of any previous observation of the unsegmented ova from the tube. Assheton's earliest specimens were already in the two-cell stage ('98). Lowrey ('11), in his study of the prenatal growth of the pig, attempted to estimate the size and weight of the mature ovum by allowing a slight addition to the diameter of the largest ovarian ovum he found, which measured 177 micra, with a zona pellucida 10 micra in thickness. He estimated, therefore, that the mature ovum would have a diameter of 180 micra. We have measured fourteen fresh tubal ova from nine sows, and find the diameter, including the zona pellucida, to vary from 155 to 165 micra, the zona being about 10 micra in thickness. The ova are plainly visible with the naked eye if placed against a strong light. We have not noticed a radial striation of the zona pellucida either in fresh or fixed ova. The ovum is filled with yolk granules of varying sizes, usually about three to five micra in diameter, which are so numerous and so refractile that they quite conceal the nucleus. A polar body may often be seen very clearly.

The ova are usually naked, but may be covered by the cells of the corona radiata, or even by a considerable portion of the discus proligerus.

A few of the ova which have been sectioned, seem to show no deviation from the usual process of maturation in other mammals; the first polar body and the second polar spindle are formed in the ovary, and the second polar body seems to be formed after fertilization. In each of two sows killed on what we believe to be respectively the sixth and eleventh days after the onset of oestrus, we were surprised to find a degenerating ovum in the tube.

In three sows in which copulation had occurred, fertilized ova were found. They all chanced to be in the same stage, just before fusion of the two pronuclei, and are therefore the earliest embryos of the pig yet reported.

The ovaries and uteri of these animals are naturally of the greatest interest, and studies of them are now in progress.

SUMMARY

1. In the domestic sow, ovulation occurs during oestrus, probably on the first or second day.
2. Ovulation is independent of coitus.
3. The mature unfertilized ovum of the sow measures 155-165 micra in diameter, has a zona pellucida about 10 micra thick, and a yolk heavily laden with fat.
4. Fertilization of the ovum occurs in the Fallopian tube, as in other mammals.

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INTRA-UTERINE ABSORPTION OF OVA

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From the Division of Anatomy of the Stanford Medical School

SEVEN FIGURES

While collecting embryological material for other purposes a decade since, my attention was not infrequently arrested by the presence of a degenerating or retarded sheep embryo in an apparently normal uterus the other horn of which contained an apparently normal foetus. Although these embryos were not infrequently quite normal in form they were always decidedly smaller than the normal embryos. In most cases the conditions suggested pathological changes both within the uterus and the embryo. The amniotic fluid was sometimes intensely turbid and even milky and more frequently dark in appearance and very evidently contained degenerating blood. Since twin pregnancies are not so very frequent in sheep, the number of cases seen was necessarily small. In uteri containing normal foetuses in the early stages of development, the embryo, the development of which had been arrested, was often represented by a rather firm fleshy mass of regular form which sometimes showed unmistakable evidences of degeneration even upon inspection with the unaided eye.

While engaged in the determination of the curve of prenatal growth in the guinea pig, Draper and myself found several cases of abnormal or at least regressive ova. All these abnormal guinea pig ova were much smaller than the normally developed ones of corresponding age should have been. Indeed, most of them were represented by firm oval fleshy masses, some of which possessed protuberances which made them look bicornuate. All were fastened with one end in more or less distinct uterine crypts which were especially evident in one of the smaller ova. The

instances met with so far were seen in pigs killed 19, 25 and 37 days after coitus.

In the first case that of guinea pig No. 16 only a single ovum was found present twenty-five days after coitus. This ovum was contained in the distal extremity of the right horn and was surrounded by a reddish black fluid. The uterus and adnexa appeared wholly normal to the naked eye. The ovum was composed of a slightly oval fleshy mass only 5 mm. in diameter although the normal embryo of this age measures 17 mm. It had a smooth regular surface and was still attached to the opened uterus but was easily detached. No placenta or foetal membranes were recognizable and the ovum protruded freely into the opened uterine cavity being attached to the uterine mucosa by its base with its longest diameter perpendicular to the latter. The line of attachment on the ovum apparently formed about one-eighth of its total perimeter.

On sectioning, the tissues of this ovum were found to be decidedly degenerated, the outer layers being composed of nothing but cell detritus. A little beneath the surface, this cell detritus is mixed with degenerating mesenchyme and variously-sized, better-preserved epithelioid cells. Between the latter lie large numbers of erythrocytes. These are scattered about freely and occupy other areas almost exclusively. Polykaryocytes and megakaryocytes in various stages of degeneration and different forms of leucocytes are also present. Some of the giant cells contain very bright golden pigment some of which is found also extra-cellularly. The framework of degenerating embryonic connective tissue, contains scattered cells and groups of cells with extremely large vesicular nuclei and prominent nucleoli.

Deeper beneath the surface remnants of blood vessels and of a reticulum which reminds one of that in young lymph nodes can be seen. In some areas, however, nothing but the degenerating reticulum with a little granular detritus remains. In addition to the giant cells large irregular masses which look like fused giant or other cells are also scattered through the specimen.

Sections made through the middle of the ovum show that the portion nearest the area of contact with the uterine mucosa is

best preserved and composed of a syncytium-like mass in which large vesicular nuclei predominate. This portion also contains a large vesicle lined by a low embryonic epithelioid syncytium. The spherical vesicle which in its largest portion comprises more than one half the diameter of the ovum contains nothing but a transparent fluid. Similar much smaller vesicles are also scattered about throughout the rest of this portion of the ovum some lying isolated at its very perimeter. A similar low epithelioid layer with indistinguishable cell boundaries also covers a portion of the surface of the most degenerated distal portion of the ovum where it also clothes villus-like extensions from the main mass. Some of the sections are almost surrounded by this epithelioid layer.

Small areas of these sections are practically devoid of tissue and contain almost nothing but a faint reticular network enclosing a slightly granular detritus and many polymorphonuclear leucocytes the nuclei of which have a typical horseshoe shape and the protoplasm of which is acidophile. Some of these leucocytes look decidedly degenerate and none seem to be phagocytic. In other often adjacent areas, the place of the polymorphonuclear leucocytes is taken by somewhat large cells with a vesicular nucleus which is circular in outline. The protoplasm of many of these cells is acidophile but here and there groups which look bright golden are seen. Most of these cells are well-preserved but some of them can be seen to be filled with similarly staining erythrocytes and what look like fragments and granules of erythrocytes.

Specimen No. 17 taken from a pig pregnant 19 days contained three ova, a normal one in the left horn and two abnormal ova in the right. The normal embryo weighed 35 mgm. and the smaller of the abnormal ova was approximately as large as the placenta and membranes of the normal embryo which weighed 0.91 mgm. The larger ovum was bicornuate. Both were single masses and no distinct placental portion was recognizable.

Both these ova which were no larger than a normal embryo of this age, were regular in form and their surfaces smooth. Upon microscopical examination, however, a few small, villus-like ex-

tensions were seen on the distal portion. A few small indentations were also evident but nothing else interrupted the regularity of the rest of the surfaces. The larger of these two ova was very well-preserved and much more vascular than that from pig No. 16. It was covered throughout by a low epithelioid syncytium which evidently was originally composed of a low cubical epithelium for here and there cell outlines are still faintly visible or the free surface of the syncytium is indented quite regularly so as to look crenated (fig. 1). These crenations are evidently the result of projections formed by the individual cells with the indentations located in the region of the former cell boundaries. The slightly irregularly-shaped, evenly-staining nuclei are thickly packed and although the cell boundaries are not clearly recognizable the layer is low and in places contains indistinct lines which look like remnants of cell boundaries and justify one in characterizing the cells as cuboidal. The rest of the ovum is composed of a syncytium containing large vesicular nuclei as shown in figure 2. Cell boundaries are distinct nowhere but the tissue apparently was a large-celled mesenchyme originally. Only a few small areas of almost complete degeneration are present. The tissue is densest and least vascular near the region of attachment to the uterine wall. The most rarefied tissue is found in the two cornua and near the distal portion where the loose mesenchyme contains small bloodvessels. Immediately beneath the investing epithelioid layer the specimen is completely canalized by wide capillaries which form an exceedingly vascular peripheral layer. A bit of the less vascular portion is shown in figure 3.

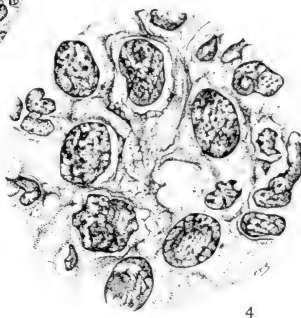
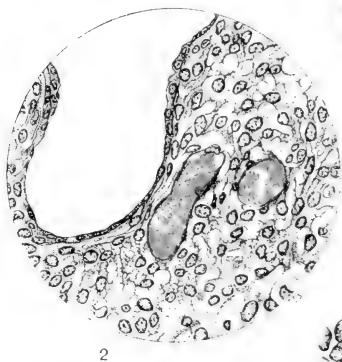
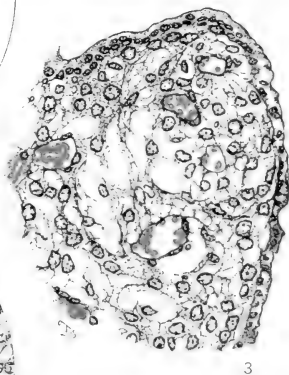
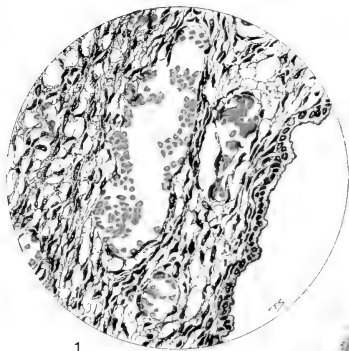
This specimen contains no large cavities but numerous smaller epithelioid-lined spaces are found in the cornua and the distal

Fig. 1 Structure of a portion of the periphery of a nineteen-day ovum.
× 475.

Fig. 2 Structure of a portion of the periphery of another ovum of the same age. × 515

Fig. 3 Structure of the vascular portion of the ovum shown in figure 2.
× 475

Fig. 4 Large nuclei from the central necrotic area of one of these ova.
× 475



portions. The largest cavity which is lined by a rather low degenerated epithelioid layer is found in the center of the specimen but it is so small that it is not visible with the naked eye in the stained section. Although better preserved these cavities or cysts are similar to the very large one contained in the previous specimen. In spite of the condition existing in this specimen the blood is all contained in vessels only a few of which can be distinguished as veins or arteries. Some of the small arteries which are located in the basilar portion of the ovum near the uterine attachment have become completely obliterated. The portion of the ovum near the area of attachment is almost non-vascular as in the previous specimen. In some of the outer portions, however, and also near the placental attachment the tissue is of a more fibrous nature and looks far less embryonic. The blood cells are well-preserved and all the leucocytes have round vesicular nuclei in contrast to those found in specimen 16.

The second specimen from No. 17 was somewhat smaller and without cornua but it also was surrounded by an abundantly nucleated syncytium and was canalized beneath its surface by numerous capillaries as shown in figure 1. Portions of the surface were also pitted by crypts which gave the periphery of the sections a fenestrated appearance. All these crypts and vesicles are lined by a similar syncytial layer and all are empty. The specimen like the previous one is most vascular near the surface and near the fenestrated portion where the tissue composing it is also much looser. Only a few villus-like processes are seen in the distal portion.

Although half—apparently the proximal half—of this specimen was lost, the structure of the remaining half is practically the same as that of the previous ovum. Its preservation is not quite so good, however, for it contains partially necrotic and small liquefied areas in its interior. The more necrotic portions of this ovum contained nuclei truly gigantic in size. This will be evident on comparing the magnification of figure 4 with those in 1 and 3. It too is quite vascular and some of the vessels all of which are full of blood, are extremely large. Some portions of this ovum look more like fibrous mesenchyme others

more like sarcomatous tissue, but cell outlines are nowhere evident.

Specimen 19 in which the period of gestation was twenty-six days contained five abnormal ova, two in the left and three in the right horn. All of these were quite equally-sized, irregular masses but they were only about two-thirds as large as a normal placenta with that duration of pregnancy. They were easily detached and projected freely from the opened uterine cavity as had those in the previous cases. In all except one ovum the placental crypts were very shallow fossae but this specimen was contained in a definite funnel-formed uterine crypt about 3 by 3 mm. in size. Since these crypts were not noticed until the ova had been removed from the uterus I am inclined to think, however, that they were formed mainly by the post mortem contractions of the uterine musculature. This assumption is also suggested by the fact that all these ova completely fitted the lumen of the uterus and formed slight elevations on its surface. No placental portion was recognizable with the naked eye and there was no gross evidence of pathological changes in the uterus.

Although these five specimens of abnormal ova varied somewhat in size this variation was not marked. All were from 4 to 6 mm. long and 2 to 4 mm. thick and in contrast to the preceding specimens the four ova which were removed from the uterus had a dull fuzzy instead of a smooth shiny surface. They were exceedingly soft and rather irregular in shape. The contracted uterus which looked entirely normal was nodular in consequence of the enlargement opposite the ova. It contained no exudate and upon microscopical examination the ova were found well-preserved. All these specimens were but slightly vascular, the small capillaries being located mainly in the peripheral layers as before. They were all devoid of an outer epithelioid layer and were composed of a syncytium containing large nuclei none of which were nearly as large as those found in the preceding specimens, however.

As in the previous specimen the largest nuclei were found in the interior of the ova and the smallest at the surface where they were more elongated and where the syncytium took on a more

fibrous and stratified character because the tissues and the long axes of the nuclei, were arranged parallel to the surface. In one portion of one ovum the extremely large cells with their large oval nuclei are still preserved and give one a good idea of what the original structure of the ovum, in the early stages of degeneration really was.

No other type of cell was found except that the formation of the giant cell masses is indicated through coalescence of adjacent degenerating cells. The capillaries are engorged with erythrocytes and a few leucocytes with vesicular nuclei, but no vessels larger than capillaries of the ordinary calibre are present anywhere. The structure of these ova at the region of the uterine attachment corresponds to the rest.

One of these specimens still shows a little of an epithelioid covering in two very small places. In one of these the epithelium is shown in the form of a tube which may represent the remnant of a crypt or an invagination. Although this ovum is completely canalized by a plexus of fine capillaries near the periphery it contains no larger vessels in its interior.

One of these five abnormal ova from No. 19 was left *in situ* and cut serially in paraffine. It measured 5.5 mm. in diameter after fixation and completely filled the uterine cavity except in a few areas where small spaces were left between the ovum and the uterine wall. A cross section of the uterus and the contained ovum measured 6.5 mm. The musculature of the uterus was thickened nowhere, there were no indications of invasion of it by the tissues composing the fleshy mass nor was there any indication of cellular infiltration. Under low magnification the uterine mucosa was evident nowhere, however, and as seen in figure 5 there seemed to be no definite line of demarcation between the ovum and the surrounding uterine wall. The central portion of the fleshy mass was less dense and contained a relatively large irregularly-shaped degeneration cavity which contained what looked like a remnant of the embryo some portions of which were in direct contact with the surrounding tissue. The whole ovum was quite uniform in structure, however, though its vascularity varied somewhat.

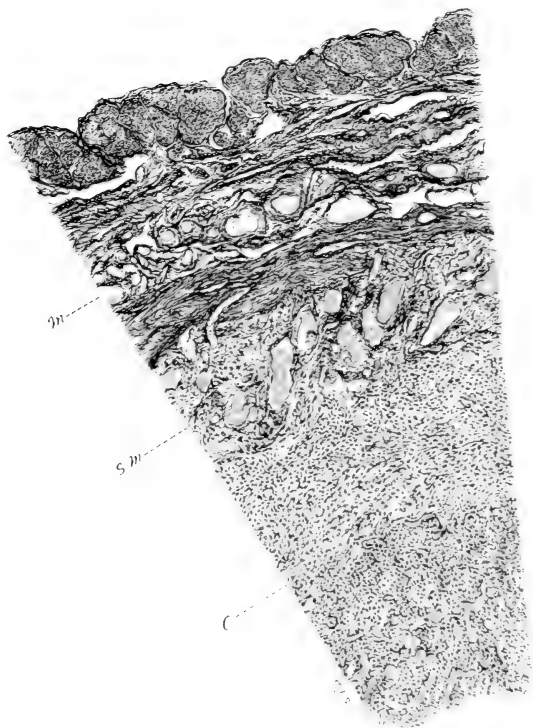


Fig. 5 Uterine wall with a portion of the periphery of an ovum of twenty-five days. $\times 1340$

Under higher magnification it was seen that glands of the uterine mucosa were present in a zone of loose mature connective tissue which probably took its origin from the submucosa and the inner portions of which were mingled with the tissues at the periphery of the ovum. The latter were also fibrous in character but much looser and more vascular and looked quite like normal embryonic tissues. They stained more with orange G,

however, while the maternal submucosa which contained some uterine glands stained deeply with fuchsin.

The outer portions of this ovum also, were composed of a loose fibrous connective tissue containing numerous capillaries filled with blood the erythrocytes of which were well-preserved. The thickness of this outer thin layer varied somewhat and from it to the center of the mass there was a gradual transition to large epithelioid cells which were plainly necrotic in places around the cavity which contained a remnant of the embryo. The latter was represented by very irregularly-shaped, folded hollow tubes composed of one and two layers of epithelioid cells which also showed signs of degeneration and apparently represent the ectoderm and entoderm. In one portion, shown in figure 6 *a-65* and *a-101* an indication of the mesoderm also seems to be present.

In some portions this embryonic tube was two-layered being composed of an outer layer of cubical and an inner of polygonal cells with large vesicular nuclei as shown in figure 6 *a-1*. In other portions the order of these layers seems to be the reverse. There were no evidences of phagocytosis and giant cells were not seen.

The next specimen was obtained from a guinea pig killed thirty-seven days after coitus. There were four fetuses, two in each horn. The distal one in the left horn was very evidently considerably smaller than the other three. The three large unopened apparently normal specimens weighed 12.2, 13.1 and 13.1 grams and the respective embryos measured 4.3, 4.5 and 4.4 mm. The fourth specimen which was abnormal weighed only 5.1 grams. A remnant of the foetus seemed to be contained in what looked like the greatly folded and collapsed membranes. The placental disc measured 1.5 cms. in diameter as compared to the normal ones which measured 2 cms. From these measurements and also from the weight of this intact specimen it is evident that the embryo in this ovum must have degenerated almost completely. This inference is also borne out by the microscopic examination. But the most interesting thing was the fact that abortion had not occurred.

Moreover, the fact that this specimen was contained not only in what appeared to be a perfectly normal uterus but was implanted within less than 1 cm. of a perfectly normally developed embryo is equally interesting and significant. The latter was as heavy and practically as large as the larger of the two embryos in the other horn. Huber '15 also found very young abnormal rat

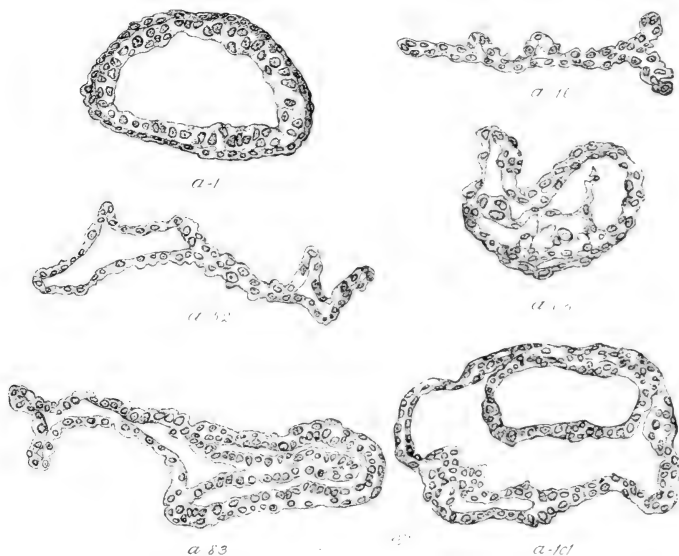


Fig. 6 Sections from an embryo contained in the ovum a portion of which is shown in figure 5. The numbers under the illustrations represent the number of the section which was drawn. The sections were cut $10\ \mu$ thick. $\times 475$

ova side by side with normal ones in apparently perfectly normal uteri. Although the abnormal ova found by Huber were very much younger than the specimen here recorded, the significance of the facts may be the same.

An incision made through this fixed specimen showed a necrotic hemorrhagic area in the center of the u-shaped mass of the

fixed placenta. Upon microscopic examination the most striking thing was the remarkable phagocytic activity in the center and the entire absence of comparable phenomena in the periphery of the placenta. There is an entire absence of phagocytosis and of hemorrhagic areas here although the nuclei in the mesenchyme are extremely large and degenerate.

It is as if absorption of this embryo and placenta were taking place from the interior of the specimen. The portion of the placenta directly beneath the embryo is decidedly hemorrhagic

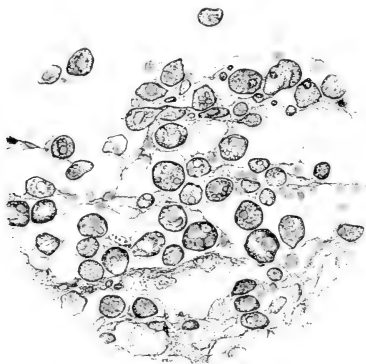


Fig. 7 A portion from the degenerating area of the placenta directly beneath the remnant of the ovum showing the remarkable number of macrophages. $\times 515$

and very necrotic. Many of the large mononuclear phagocytic cells found in the central area and shown in figure 7, are so degenerate that they are mere shadows. Others are full of vacuoles and still others of erythrocytes and cell detritus. Many of them look so necrotic that one must doubt their ability to have remained actively phagocytic much longer even if they possess cell inclusions. The nuclei of the larger cells are usually small and not very evident but in the small cells which possess fewer or no cell inclusions they are relatively larger and are also stained better. Most of these macrophages have an acidophile

protoplasm. Although much blood is contained in this necrotic area only very few polymorphonuclear leucocytes are seen and these do not show evidence of phagocytic activity. In some areas these large phagocytes lie in a wide-meshed reticulum. For a discussion of the origin, relation and properties of these cells the reader is referred to Evans '15.

From figure 6 it is quite evident that although these ova may have developed normally up to a certain point they must subsequently have developed abnormally. The disproportion between the size of the embryo and the placenta alone shows this. Furthermore, since the very early rat ova described by Huber already showed degeneration phenomena it follows from this as well as from the relatively large size of the placenta that the life of the embryo must have been prolonged for a considerable period of time.

The cause of death of these ova must, to be sure, remain a matter of conjecture although the gross and microscopic character of the uteri would seem to indicate that the cause probably was intra- rather than extra-embryonic if it was not due to defect in the corpora lutea. At any rate death of the ovum did not seem to be due to a defective placental development although it must be borne in mind that it is possible even if not probable, that the uterine site upon which implantation occurred may nevertheless have been pathological. Such an assumption is made very unlikely, by both the apparently normal development of the placentae and by the surprisingly extensive development of the latter. The latter fact also seems to indicate that the early placenta even possesses considerable independence of the embryo.

Although the question as to whether or not abortion would finally have occurred in these cases must remain a matter for conjecture, I am ready to believe that such a termination would not have occurred. To be sure, such an assumption presupposes the gradual absorption of these ova, embryos and placentae and also raises the question as to what percentage of pregnancies terminate spontaneously in this way even after considerable development has occurred. If such a regression and

absorption occur in man also the surprising percentages of spontaneous terminations of pregnancies given by Mall '08 and '10 would be increased still further.

It will be recalled that Frankel '03 was able to cause death and intra-uterine absorption of ova in rabbits up to the twentieth day of pregnancy through destruction of the corpora lutea. Frankel found that abortion did not occur in these rabbits and described the gross changes as follows. The egg-chambers which became less tense because of a decrease in the production of amniotic fluid also became folded longitudinally, wrinkled and changed in color from a very red to a pale yellow. Frankel considered the reduction in the quantity of amniotic fluid as the first sign of regression and noted that the spherical chamber became more elongated, cylindrical, firmer and nodular. The embryo also became drier, smaller and more unrecognizable, finally being dissolved and represented only by an amorphous grayish white 'Schmiere.'

The placenta was preserved the longest and could be recognized as such for several days later. But it also became dry and pale red, only a few fragments finally remaining in the longitudinally folded and slightly swollen mesometrium. In this stage the uterus was only slightly firmer and showed but a minimal enlargement. After fourteen days even these evidences of a past pregnancy had disappeared only an anemic ring remaining and after three weeks not the least indication was left of the interrupted pregnancy.

Although the intra-uterine absorption of all guinea pig ova belonging to a single pregnancy could be due to defective development of the corpora lutea or entire lack of development of the latter it is much more difficult to see how regression and absorption of a single ovum lying between apparently normal ova could occur. It is conceivable, of course, that the growth of the corpora lutea might be sufficient for the development of four and not for six or more ova but one would expect all to suffer a corresponding retardation rather than have one or two destroyed and the rest preserved.

Since the ovaries of the guinea pigs concerned in these incidental observations were unfortunately not preserved I am unable to report on their condition. Nevertheless, even this small series of cases indicates that intra-uterine absorption of ova is not a rare phenomenon in guinea pigs. This conclusion is also in entire accord with Frankel's observation regarding rabbits. According to Koebner, Frankel concluded that the physiological regression of one chamber is very common, and emphasized that in many cases not all fetuses reach normal development. Individual fetuses die and are resorbed together with the placenta, while the rest go on to maturity. From these observations of Frankel which so far as I can learn were drawn from experimental work, Koebner observes that the rabbit is apparently less disposed to abortion in the first twenty days of pregnancy than to a "dry degeneration and resorption" of the fetus. Koebner '10 found that the bones also are absorbed under experimental conditions in the rabbit, and Williams '16 while discussing the subject of missed abortion in women states that "In very exceptional instances the entire product of conception may be absorbed without a sign of external discharge. Polano and L. Frankel have reported cases in which this occurred after the pregnancy had advanced as far as the fourth month and Koebner has demonstrated its possibility by animal experiments."

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METHODS OF MOUNTING SECTIONS IN GELATIN

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Gelatin has been used to some extent by various European workers for mounting sections, but has not come into general use. Our experiments show that gelatin possesses three properties which render it undesirable for this purpose until overcome by special treatment. Gelatin is non-hygroscopic, brittle and inelastic, and unstable. In the extremely dry winter climate of Minnesota the gelatin dries and shrinks until it bends or breaks the glass slide on which it is spread, or cracks and peels off the slide. In very moist summer weather the gelatin may become whitish-opaque from moisture. When these qualities are corrected by the addition of glycerin to render the film hygroscopic, sugar to make it elastic and pliable and some hardening agent to render it stable, a satisfactory and permanent mounting medium is secured. To render the gelatin insoluble in water, formalin, chrome alum, chromic acid, tannic acid or potassium bichromate may be used with varying results. The most satisfactory product is obtained by means of potassium bichromate or chrome alum. After the addition of one of these salts the gelatin is rendered insoluble upon exposure to light. There is also produced a very slight grayish-green tint by the chrome alum and a deeper greenish tint by the bichromate, but this does no harm in thin films. Perhaps the best results are obtained by protecting the gelatin by means of an insulating varnish. The varnish seals the gelatin against the action of atmospheric vapor and also prevents the evaporation of water from the hardened gelatin film, the presence of water being necessary to maintain elasticity and pliability.

The advantages of a gelatin mounting medium are: saving of expense of dehydration for mounting in balsam and of the cost of cover glasses, and availability in some cases where an aqueous medium is necessary. It can be used on sections stained with haematoxylin, carmine and some but not all of the anilin dyes (e.g., not with acid stains soluble in water). Its use in films without glass may prove to have some value.

SOLUTIONS TO BE EMPLOYED

- A. Best quality photogelatin 5 grams.
Distilled water 100 cc.
Add glycerin 5 cc. Let stand two hours.
Raise to 50°C. Gelatin dissolves.
Filter through cotton flannel.

- B. Hydrate 2 grams, gelatin in 45 cc. distilled water.
Add 10 cc. glycerin and 15 cc. corn syrup.
Raise to 50°C.
When gelatin is dissolved add 18 grams gelatin hydrated and dissolved in 55 cc. distilled water.
Filter through canton flannel.
- C. Prepare as in B, using 2 grams gelatin and 45 cc. water and 23 grams gelatin and 55 cc. water.
- D. To render A, B, or C insoluble add to the solution prepared as above $1\frac{1}{2}$ cc. of a 10 per cent solution of potassium bichromate or chrome alum to every 100 cc. of the gelatin solution.
- This solution must be used at once, since it will not melt after being allowed to harden.

In making the above solutions a temperature of 45° to 48°C. is necessary to dissolve the gelatin. After the gelatin is dissolved the solution may be lowered to 30°C. without causing the gelatin to set. In mounting sections it is necessary to keep the gelatin sufficiently warm to secure penetration, but advantage should be taken of the fact that the gelatin remains fluid at lower temperatures which are less likely to harm the tissues. In the following directions it is intended that the gelatin itself shall be kept at about the temperatures indicated, whether by means of an oven or a constant temperature plate.

For paraffin sections

Bring into distilled water on the slide. Place slide on constant temperature plate at 35°C. and cover with sufficient solution A to make a complete covering film when dry. After a few minutes on the warm plate set in a horizontal position to harden.

For celloidin sections up to a thickness of 100 microns

Carry the sections on paper ('onion skin' best). Clear in glycerin and water over night, followed by glycerin several hours.

Immerse sections in solution A in flat dish at 35°C., 20 minutes. Clean and flame¹ a slide and place on warm plate. Spread on slide a small amount of solution A and immediately place section on it, removing the paper.

Carefully press out all air bubbles.

Add more solution A and then drain to secure a thin but complete covering for the section.

Keep on level warm plate until gelatin is evenly spread.

Place in horizontal position to dry at room temperature.

¹Flaming the slides is necessary to remove the thin film of organic material which is taken up from the air by slides exposed for any length of time. The usual cleaning solutions do not wholly remove this.

To secure a sufficiently thick and even covering, when the five per cent gelatin is used it is best to make the first coat thin and apply a second and third coat to the slide when cold, draining each time.

For thicker celloidin sections (1 to 2 mm.)

After clearing in glycerin immerse the sections for at least three hours in solution A at as low a temperature as practicable (30°C.) and mount in solution B by the method just given.

For mounting sections in films without glass

Fasten together two very thin celluloid films by means of snaps on one edge, place the section or sections between them and immerse in water or glycerin at 35°C. Drain, separate the films and sections as they are immersed in solution B in a flat dish on warm plate. Abundance of gelatin solution must be used, to secure complete immersion. After twenty minutes carefully press out the air bubbles with the fingers or a rubber wedge. Attach snap hangers to all four corners of the film and hang up to dry at room temperature. The hangers at the lower end serve as weights to prevent warping.

For thick sections (1 to 2 mm.) in films, immerse first in solution A at least three hours and then mount as just directed, using solution C. As this thick solution does not drain off readily, the excess gelatin should be removed from the surface by stripping the film between thumb and finger.

Solutions A, B, and C may have thymol added and be kept cold and remelted when wanted.

Finally, in each of the above cases either an insoluble preparation (solution D) should be used or the finished preparation should be covered with a varnish, as soon as it is dry enough not to be sticky. One may use the Zapon varnish found in the market. The same may be made by dissolving 5 grams celluloid in 20 cc. amyl acetate and 80 cc. acetone. A good varnish which is more pleasant to use is made by Abney as follows:

Alcohol 20 ounces
Ether 40 ounces
Pyroxylin or celloidin 400 grains

In applying the varnish care should be taken to have the gelatin completely covered. Films should be dipped in the varnish, and on slides the varnish should be spread with brush or spray beyond the edges of the gelatin.

December 8, 1916

THE VALUE OF ABSOLUTE ALCOHOL FOR REMOVING ADHERENT PARAFFIN SECTIONS FROM PAPER OR PASTEBOARD TRAYS

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Laboratory workers in embryology, histology, and neurology, and especially those whose work calls for the mounting of serial paraffin sections, have all at some time been annoyed by the adhesion of their sections to the paper or pasteboard tray on which they had been placed prior to mounting. This occurs, of course, most frequently during the warmer months of the year or where sections are not mounted for a long time; thus leading often to a complete loss of a valuable series.

Because of this it may be of value to call attention to the use of absolute alcohol as a means for overcoming this difficulty. The amount of alcohol used is very slight, being just sufficient to moisten the sections completely and in addition overrun on the sides so as partially to impregnate the paper or pasteboard to which they adhere. After this, several minutes time should be allowed for evaporation of the alcohol. This requires only a brief period, but can be hastened if a current of cool air is allowed to strike the tray. Where the sections adhere with especial firmness this last is particularly desirable.

It is of advantage when dealing with large sections to take a section-lifter or fine-bladed scalpel and run it along under the edge of the sections before the alcohol has completely evaporated. After this procedure sections which adhered firmly loosen almost always with ease.

The use of 95 per cent alcohol is not recommended since with it satisfactory results are less often obtained. It does not evaporate with the rapidity and thoroughness of the absolute alcohol. The latter vaporizes very readily and to this its action of loosening paraffin sections from paper or pasteboard trays may possibly be ascribed.

¹ Contribution No. 47, November 1, 1916.

PRESERVATION OF ANATOMIC DISSECTIONS WITH PERMANENT COLOR OF MUSCLES, VESSELS AND ORGANS

A SUPPLEMENTARY NOTE, DESCRIBING ANOTHER METHOD, THE CURING METHOD

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Since the printing of the original manuscript in the *Anatomical Record*, Vol. 10, No. 1, November, 1915, I have found out a new and simpler method, the curing method. It consists in the following: After the completion of the dissection, if the muscles present a dark brown color, the preparation is immersed in G33C1 for three days. G33C1 means a solution composed of 33 per cent of glycerine, 1 per cent of carbolic acid and 66 per cent of water. It registers 10° Baumé.

At the end of the three days it is taken out and exposed to the air in a room until the muscles become black. This requires about ten days. If some muscles do not blacken as fast as the others, they should be painted daily with pure glycerine until they become as black as the others.

As soon as all the muscles are black the preparation is immersed in Ch.F75, i.e., a solution of chloride with 75 per cent of formal, as previously described.

Then it is placed permanently in Ch.F5.

The results are quite satisfactory. The advantage of this method is that it does away with the use of calcium chloride, described previously, which is not always uniform.

Pale muscles do not do so well by this method. They should be painted in preference.

I have also brought out the following points which are of some assistance in the work.

In using tallow for distending the arteries, it is best to use beef tallow from around the kidneys. It is obtained from the butchers at the market. Tallow from corn fed beef is the best.

When the dissection is completed, if the muscles present a dark brown color they are suitable for the curing method. They are comparatively scarce. If the muscles show a lighter color than dark brown they are suitable for the paint method. They are much more common than the dark brown.

In the final preservation of curing preparations Ch.F5 will do as well as Ch.F.20; thus effecting a marked saving in the cost of the permanent solution.

I found that the following mixture of paint gives better results: Tuscan red, half teaspoonful; turpentine, 2 teaspoonfuls; lamp black, 2 grains. The lamp black tones down the Tuscan red when too bright in the solution. This quantity is more than enough to paint two or three times all the muscles of the upper extremity. Try the mixed paint on 1 or 2 inches of a muscle to see how the color will show and modify the paint accordingly.

When using lamp black, any dark red paint will do. Tuscan red is not so essential.

Two or three thin coats of paint are better than one thick coat. Thick coats make preparations look like daubs.

When the preparation is placed in the solution, if the color is not satisfactory, too light or too bright, do not hesitate to take it out, expose to the air for a few hours to dry and then repaint, with a suitably prepared paint. Do this two or three times if necessary. Final success depends on it.

Painted preparations do better decidedly on A10F.5 than in A10 alone. They do not require filtering as often and filter better. The F.5 prevents the bacterial cloudiness. It does not seem to affect the color of the paint any more than A10 or A20.

Artists' oil paints (Winsor and Newton) are better for the vessels than any other. Deep Vermilion for the arteries and Permanent Blue for the veins.

When the paint remains in the cup over night, even with a lid, it becomes too thick and turpentine must be added to it before using it.

A flat brush of fine bristles about $\frac{1}{3}$ inch wide is quite handy to paint large surfaces.

Keeping solutions clear all the time is the foundation of the preservation of the preparations.

When solutions have been filtered twice in succession, if they do not come out clear, they should be changed for fresh solutions.

Solutions very cloudy or very discolored filter badly. They clog the filters. It is best to make new fresh solutions.

When solutions get old (two or three years) they should be changed unless they remain clear.

THE DEVELOPMENT OF THE HUMAN CHIN

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Linneus is generally given the credit of having been the first to make the observation that the chin is a characteristically human trait. In none of the anatomical or anthropological discussions of the chin has the writer seen credit for this shrewd observation given to any earlier scientist. Observation and record of the fact that the chin is a characteristically human trait is, as a matter of fact, centuries older than Linneus, and it is doubtful whether the latter did not copy it from some older authority. Pliny in Book XI, Chapter 60, of his *Natural History*, informs us that "no animal, with the exception of man, has either chin or cheek bones." The Greeks when in the act of supplication, touched the chin to show, as some would say, their affinity with the divine, and, if this is true, making fitting recognition of its human peculiarity as a trait not shared by the animals. But no Greek scientist seems to have speculated about its origin.

The attempt to explain the evolution of this anatomical feature is certainly comparatively recent. It appears to be no earlier than Cuvier. Cuvier remarks that in certain quadrupeds, individuals occasionally are born with the upper jaw unusually small; as a result the lower jaw, by the inturning of the alveolar processes to articulate with the unusually short upper ones, gives rise to a mental prominence bearing a close resemblance to the human chin. He claims to have seen an instance of this in a calf of Geneva, of reputed human paternity. Cuvier's explanation was a shrewd one. Sir Ray Lankester has used a precisely similar argument in accounting for the evolution of the chin of the elephant which, as he has shown, has developed *pari passu*

as the jaw has shortened, the unsupported upper lip meanwhile lengthening into the trunk of the modern elephant.

In the past century considerable attention has been given to the development of the chin and various explanations of its evolution have been offered. Any discussion of the problem should give some review of these views and we have attempted to summarize them below.

SPEECH

Attention has often been called to the effect, or supposed effect of speech upon the mandibular conformation—or vice versa. Osborn, for example, reminds us that the narrow passage between the alveolar processes which, in the simia, lie in almost parallel, or, in some cases, in forwardly converging lines, give small range for the action of the tongue.¹ Whether this restriction of the play of the tongue would seriously interfere with speech, may be doubted. We speak of tongues wagging, but they do not really wig-wag so much as hump themselves while oscillating upward and downward in interfering with expiration. It is only upon failure of speech that we stick our tongues in our cheeks. The uplift in the human palate might be considered more favorable to speech than the increased width of the alveolar processes. Defects in the palate cause defects in speech, but we do not hear that people with long, narrow jaws have less linguistic facility than those with short, broad jaws.

The effect of the mandible upon speech has not, however, elicited so much defense as its converse, the effect of speech upon the mandible. In the mandible of simia will be found a deep pit lying in the inner concave surface. This seems to be absent generally in other mammals, including mankind. It accommodates the genio-glossus muscle which rises here and spreads out, fan-like, along the middle line of the lower surface of the tongue. This muscle, Dr. Louis Robinson believes² aids

¹ Men of the Old Stone Age, 100, 139-140, Scribners, 1915.

² Evolution of the chin. North American Review, Sept., 1914, vol. 200, p. 438-449.

the tongue in sorting out the contents of the mouth. The dog, for example, seems to have considerable difficulty in getting rid of undesired morsels, while cattle, the giraffe, and the camel, shift the undesired portions to one side, where long, projecting papillae help to work them out along the cheeks as they are agitated by the tongue. In place of this pit we find in the human jaw a bony prominence or tubercle, and Dr. Robinson professes to be able to trace the gradual evolution of this pit for the accommodation of the genio-glossus muscle, as we find it in the simia, to the bony prominence known as the genial tubercle which replaces it in the human jaw. The pit itself is subject to much variation, being most shallow in a fossil lemur, and shallow in the anthropoid apes, because a downward tilting of the margin of the jaw below the incisor teeth gives larger surface for the attachment of the muscle. The depression seems to be least in the siamang gibbon, while the human jaws of Heidelberg and Naulette are said to show it to a slightly less extent than the siamang gibbon. As these are among the oldest and most simian of human jaws the correspondence is not to be lightly passed over. Klaatsch³ confirms the existence of a fossa sublingualis in the Heidelberg jaw, and says that in the Hauser skull from Le Moustier no genio-glossus spinal prominence is present, its place being taken, as also in the Mauer and Krapina G. skulls, by a fossa or pit for the insertion of this muscle.⁴

The insistence of Robinson is not without precedent. In 1904 Dr. C. Toldt, of Vienna, at a meeting of the German Anthropological Society of that city, suggested that the evolution of the chin was due to the development of the muscle emanating from the tongue and necessitated by speech. He returned to this argument the following year, in a paper entitled "Über die Kinnknöchelchen und ihre Bedeutung für die Kinnbildung beim Menschen."⁵ This paper called forth a reply by von Hause-

³ *Zeitschrift für Ethnologie*, 41 ('09), 554-555.

⁴ See also Hausemann, *ib.*, p. 719, 721; R. R. Schmidt, *Die Diluviale Vorzeit Deutschlands*, 234 (Stuttgart, 1912); K. Gorjanovic-Kramberger, *Der Diluviale Mensch*, 176-181.

⁵ *Vienna Anthropologische Gesellschaft Mitteilungen*, 36 ('06), 51, 54.

mann, who insists that the ossicula mentalia are to be otherwise accounted for, and that other causes have been primarily responsible for the evolution of the chin.⁶

C. C. Blake⁷ considered the absence of the genial tubercles in the La Naulette jaw purely adaptive: "The relative and absolute great thickness of the jaw at its symphysis originates this shelf-like structure, which is solely caused by the great deposit of osseous matter around the site of the genial tubercles."

Mr. Roosevelt finds the jaw of the chinless homo heidelbergensis so primitive that it must have made his speech thick and imperfect.⁸ The veteran French anthropologist, Paul Topinard, is not impressed by such arguments. In his discussion of the significance of the absence of the genial tubercles in the La Naulette jaw⁹ he points out that only a small portion of the muscles from the tongue, namely, the geniohyoid muscle, which reaches from the small hyoid bone at the base of the tongue to the symphysis, is attached to the lower part of the tubercles, the genio-glossus muscle finding attachment to the region above this. Instead of accepting the presence of tubercles as an advantage, he insists that the depression in the gorilla's mandible gives him considerable advantage over man. Albrecht found the mandible of an idiot of twenty-one years of age possessing tubercles that attained the extraordinary eminence of 9 mm.¹⁰ This outvies the tubercles of educated orators. This idiot, observes Topinard, did not have half the persuasive eloquence doubtless enjoyed by the La Naulette lady.

⁶ See his paper on Die Bedeutung der Ossicula Mentalia für die Kinnbildung. *Zeitsch. f. Ethnol.*, 41 ('09), 714-721; see also P. Bartels in *Int. Monatschre f. Anat. and Phys.*, vol. 21, p. 179 ff.; Osborn, op. cit., 228; R. Virchow, in *Zeitsch. f. Ethnologie*, 14 ('82), 287; Schaaffhausen, in *Korr. d. Deutsch. Gesell. f. Anthropol.* (Jan. 1881), No. 1, p. 3; *L'Anthropologie*, 4 ('93), 753-754.

⁷ *Anthropological Review* ('67), vol. 5, 295-302.

⁸ *National Geographic Magazine*, Feb., 1916.

⁹ *Rev. d'Anthropologie* ('86), serie 3, vol. i, 416-25.

¹⁰ See *Bull. Soc. Anthropol. d. Bruxelles*, i ('82-'83).

DECREASE IN THE SIZE OF TEETH AND OF THE ALVEOLAR PROCESSES

When our forebears assumed the erect posture and were able to fight freely with those fists which no longer had to serve as supports when running, the dangerous canines became shorter, and there was a gradual degeneration in the size, if not in the number also, of the teeth. The large bony alveolar processes in which they lay embedded were no longer needed, and there was a corresponding shrinkage in this bony structure. The absorption of the alveolar region leaves the lower portion of the mandible, which is more solid and less liable to change, relatively prominent and suggestive of a chin.

This view has been popular. Toldt, in the paper referred to, insists that the reduction in the size of the teeth, together with the drawing in of the enfolding alveolar processes, would tend to develop the chin. His commentator, von Hausemann⁶ recognizes the force of this portion of Toldt's argument. Bardeleben¹¹ pointed out that the building up of the chin would result from reduction in the size of the teeth, and declared he knew of no exception to this correlation. In accounting for the reduction, he not inaptly likened the building of the chin to a hillock left out-standing on a plain where erosion has reduced the general level. The osseous portion which is left outstanding, like this older stone formation, becomes a protruding chin. The receding of the lower jaw is not to be forgotten. In this recession the teeth and the alveolar processes are especially involved, so that the protuberantia mentalis gradually comes to the fore—as we find even with prehistoric man. Similarly, Arthur Keith, while attributing to the muscles of the tongue a tendency to give a forward development to the chin, lays more stress on the recession of the alveolar processes that accompanies reduction in the size of the teeth.¹²

Robert Munro in 1912¹³ attributed the prominence of the chin to retrocession of the facial bones, "as the shortening of

¹¹ Anatomischer Anz., 26 ('05), 107.

¹² Man: A History of the Human Body, 193-194. No date.

¹³ Paleolithic Man, 19 (Macmillan '12).

the alveolar ridges would cause the teeth to assume a more upright setting in their sockets." Walkhoff noted the tendency of the reduction in teeth and alveolar processes to give rise to a chin.¹⁴ Weidenreich¹⁵ viewed the progressive development of the chin as purely a passive process: it got ahead by remaining where it was, the superior alveolar region being meanwhile in retreat. Rudolf Martin is inclined to champion these views¹⁶ as is also Osborn. The latter adds to this tendency the growth and specialization of the muscles of the jaw and tongue employed in speech, though he insists that absence of the chin does not betoken inability to speak. Prof. T. T. Waterman has recently added his support to this school.¹⁷ That absorption of the alveolar processes will leave the chin prominent is proved in the changes that take place in old age, where both process and result can be observed.¹⁸

Thus the changes that take place during the life of the individual are to some extent an epitome of those that are recorded by prehistoric evidence. "In all modern races of men the front part of the semicircle arch of teeth has shrunk or 'withdrawn' considerably, or more than has the bony jaw in which the teeth are set. Consequently the bone projects in front of the teeth as the bony chin."¹⁹ Robinson recognizes the argument that shrinkage of the alveolar processes gives rise to the chin, but discountenances it.

¹⁴ Die menschliche Sprache in ihrer Bedeutung für die funktionelle Gestalt des Unterkiefers. *Anat. Anzeig.*, 24 ('03), 129-139; Beitrag zur Lehre der menschlichen Kinnbildung, *ib.*, 25 ('04) 147-160; see *L'Anthropologie*, vol. 15, 99-100, 235-236 ('04). Similarly Frizzi, *Archiv. f. Anthropol.*, ('10), vol. 37, p. 255 ff.

¹⁵ Die Bildung des Kinnes und seine ungebliche Beziehung zur Sprache. *Anat. Anzeig.*, 24 ('03-'04), 545-555.

¹⁶ Lehrbuch d. Anthropologie, 873-874, Jena, 1914.

¹⁷ The evolution of the chin. *American Naturalist*, April, 1916.

¹⁸ See the description and illustration E. G. Norris, *Human Anatomy*, 65. Much valuable material will be found in the article by Ernst Frizzi, Untersuchungen am menschlichen Unterkiefer mit spezieller Berücksichtigung der Regio mentalis. *Archiv. für Anthropologie*, 37 ('10), 252-286. Contains extensive bibliography, 101 sketches, and detailed measurements of 100 mandibles of different races.

¹⁹ E. Ray Lankester, *Diversions of a Naturalist*, 250-252 (London 1915).

OTHER OSSEOUS CHANGES

The changes in the anterior portion of the mandible, if we compare that of an anthropoid with that of man, are not exhausted in the differences already noted, but include other important features. The os mentale in the simia is narrow, regular in curvature, and of smooth surface. The human os mentale often has a jagged border, and may be medially concave both in a horizontal and in a vertical plane, when viewed anteriorly. Most striking of all, it possesses, on both the right and the left side, a small bony protuberance or ossicle, which gives roughness to the contour, adds to the impression of greater breadth, and breaks up the convex lines into horizontal concave surfaces. Where normal osseous changes are not located at articular surfaces, we usually have to account for them as due, either to some shift in adjacent bony tissue which directly affects them, or to the action of muscles. The importance of the tuberosities at the symphysis in giving prominence to the chin, should not be forgotten. Their importance as a factor in the development of the chin has been recognized by B. Adachi.²⁰ Mies points out the value of the ossicula mentalia in building up the chin, and also the fact that paired muscles are associated with paired ossicles, whereas an undifferentiated muscle is associated with the undifferentiated ossicle.²¹ A similar argument was later adduced by Toldt, who attempts to show that the development of the ossicula mentalia, which proceeds along different lines in the human and in the simia, has been influential in giving rise to the chin.²²

MUSCULAR AND MECHANICAL FORCES

That these bony protuberances are directly related to muscle development is more than probable. Bardeleben's extensive investigations show that man alone possess on the os mentale

²⁰ Über die Knöchelchen in der Symphyse des Unterkiefers, *Zeitsch. f. Morph. und Anthropol.*, ('04) 7, 369-372.

²¹ Über die Knöchelchen in der Symphyse des Unterkiefers vom neugeborenen Menschen. *Anat. Anzeig.*, ('93), 8 p. 361-356.

²² Die Ossicula mentalia und ihre Bedeutung für die Bildung des menschlichen Kinnes. *Sitz. d. Kaiser. v. Akad. d. Wiss.* ('05), 114, (AB. 111), 657-92.

the paired (paariger) muscle, the *musculus anomalus menti*, which is associated, whether as cause or effect, with this bifurcation of the *os mentale* in the human. Not only is there this difference between *simia* and *homo sapiens*, but, as a consultation of G. Rugge's, *Die Gesichtsmuskulatur der Primaten*²³ will show, considerable differences in the muscular system of the two are represented in this portion of the face.

If the hand is held on the collar bone, the chin tilted in air, and the skin covering the chin raised as far as possible, the hand will detect movements over the surface of the clavicle. These movements are effected by a large muscle, or system of muscles, which rises in the lower lip, passes over the *os mentale*, down the front of the neck, and over the clavicle and some of the upper ribs. In the *prosimia* a large bundle of *platysma* pass over the *os mentale*, due partly to the narrowness of the anterior margin, partly to the demand for larger muscles in the larger lips which do much more heavy work than the human lips. The tendency wherever muscles pass over a bony surface and pull against it, is to flatten that surface. Hence the regular rounded contour of the *os mentale* in the *simia*, with absence of outstanding bony prominences such as we find in the human mandible.

There is, moreover, in the human, a specialization of muscular development which has proceeded far beyond that of the apes. The *os mentale* is traversed also by a set of muscles known as the *musculus mentalis*, which run at almost right angles to the *platysma*. In the human face these systems are separate and specialized, while in the *simia* they are often so interwoven as to make it impossible to entirely separate them. In *Ateles*, for example, it is difficult to distinguish parts of the *mentalis* from the *platysma*, while in the chimpanzee the complication is even more marked. Similarly, according to Robinson, there is both greater development and greater specialization in the *genio-glossus* muscle in human beings than is to be found in the apes. "In man the *genio-glossus* has become a series of a large number of independent muscular strips which are to all intents and purposes separate muscles, each with its little fiber of the hypo-

²³ Leipzig, 1887.

glossal nerve entering it in such a way as not to hamper its free movement, while in the apes it is apparently a single muscle, or a closely united group, acting en bloc."²⁴

It has been already mentioned that the paired muscle on the os mentale is found only in human beings, and that it seems to be related to the external genial tuberosities peculiar to the human os mentale. Rugge noticed that the mental region of the human skull is distinguished from that of the simia by the possession of numerous tubercles which must be supposed to be the points of origin for many small muscles not to be found in the apes.

We need only consider the comparative range and facility of facial expression in the two species to grasp both the fact and the explanation of the existence of these numerous tubercles that cover the human mandible and their absence in the simia. The rapid pull of facial muscles used in articulation may have contributed not a little to this result. Some of the muscles used in such facial twitchings as laughter, for example, involves, find attachment on the anterior surface of the mandible. Here the bony prominences that rise to give these muscles attachment help in the outer construction of the chin. No one who has read the detailed account of the muscles used in facial expression, given by Prof. Arthur Thomson in his *Anatomy for Art Students*, can be skeptical about the much greater specialization of facial muscles in the human being and the tendency of these to elicit bony prominences on the skull and the mandible as points for attachment.²⁵

It is not improbable that this difference in muscular pull will do much to explain the simian type of chin. The thick-lipped peoples, as notably the Negroes, have, of course, larger muscles to work their larger lips, and they have less prominent chins. Those negroid peoples who have thinner lips have more prominent chins. The chin is well developed in the Eskimo, though they possess a long and heavy mandible, and large teeth. In the typical negro the chin is but feebly developed, in keeping with

²⁴ An. Rep. Smith. Inst., 1914, p. 603; Knowledge (London), Nov., 1913.

²⁵ See also Alfred Fripp, *Human Anatomy for Art Students*.

the heavy, thick, protruding lips, though the negro mandible is not larger, longer, nor heavier, nor are the teeth larger than those of the Eskimo. In the more orthognathous Bushman, with smaller, thinner lips, we find, on the contrary, a well developed chin of the anteriorly concave type. There is, in fact, a general, though not a complete, correlation between thick, prominent lips and retreating chins.

The protruding teeth which are associated with the retreating chin, enable the animal to get rid of the food with comparative ease. In fact, if the teeth were set upright, as are ours, to disgorge would be fraught with considerable difficulty. If this advantage of being able to get rid of unwelcome and retain desired contents is to be preserved, large, long, strong upper lips are needed in a land where many hungry mouths linger round to pick up a fallen morsel or snatch a disappearing one. Our remote ancestor, 'probably arboreal,' had to keep a tight under lip until he could carry his head as high as the descendant who became lord of all he surveyed. Then large under lips were no longer necessary. In our contemporaries they serve merely to proclaim the proximity of their simian ancestry.

Mr. G. F. Scott Elliot asserts that in consequence of the broadening of the skull and the shortening of the face into a shorter, rounded-arch shape, great strain and cross-tensions would be thrown on the extreme forward points of the jaw-bones.²⁶ In this I do not follow him, for it seems to me the tendency would, if anything, be the opposite. Nor do I follow him in his suggestion that if the origin of bone-forming tissue may be due to muscular stresses and strains, then the production of bone-forming tissue at the chin would be favored—unless he means such stresses and strains as we have indicated. So far as mastication is concerned, the muscular stress would be much greater in the simia, which use their heavy, protruding lips in lieu of free hands, to pull in their food, and certainly when eating, use them much more than we use ours. But the masticatory muscles are not attached to the chin and could scarcely affect the *os mentale* directly. Simia must do more

²⁶ *Prehistoric Man and His Story*, 76-77; (London, 1915).

severe work with canines and incisors than we do with ours, but the muscular strain comes much further back on the mandible than Elliot supposes.²⁷

The muscles used in deglutition must, of necessity, be larger and stronger in the simia than in human beings, and, so far as they attach to the anterior part of the mandible, we might expect them to exert some influence on its conformation. That these muscles do exert an influence on the shape of the chin is more than probable. Bijvoet, in a detailed study of the morphology of the *musculus digastricus mandibulae* in the zoological world, including man and the primates, demonstrates its varying area and method of attachment, which is not the same in those animals which chew with a scissor-like motion in the vertical plane, as in those whose jaws move with a side to side grinding motion—differences which are to some extent typified in man and the monkey, Duckworth's assurance to the contrary notwithstanding.²⁸

CHANGES IN HEAD FORM

The lower jaw is not anatomically a part of the skull, yet it would be wrong to suppose that we can consider it as a feature isolated from the remainder of the head, since, physiologically, it is an integral part of the head. The upper alveolar processes are the supplementary portions which make, with the mandible, a functional unit. The various portions of the skull are so closely interrelated, either structurally or functionally, that any considerable change in a given region is apt to be reflected by

²⁷ The method of muscle attachment has been well shown by C. Toldt, *Der Winkelfortsatz des Unterkiefers beim Menschen und bei den Säugetieren und die Beziehungen der Kaumuskeln zu demselben*. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, 1905, vol. 114, (Abteilung III), 315–478. Further descriptive and illustrative account of the differences between the human and the simian will be found in C. Toldt's papers, *Der vordere Bauch des M. digastricus mandibulae und seine Varietäten beim Menschen*; and, *Der digastricus und die Muskeln des Mundhöhlenbodens beim Orang.*; Sitzungsberichte der Kais. Acad. d. Wiss., 1907. Ab. III, vol. 116, p. 373–459; vol. 117 ('08) p. 229–324.)

²⁸ Bijvoet, *Zeitsch f. Morph. und Anthropol.*, 61 ('07–08), 249–315. W. H. L. Duckworth, *Anthropology and Morphology*, vol. I ('15).

corresponding or compensating changes throughout the entire skull.²⁹

The upper alveolar region is an incorporated portion of the facial skeleton, and is dependent for its evolution upon changes in the facial region. The face is, in turn, architecturally but a pendant portion of the upper supporting skull case, so that changes in the skull case, affecting the points of juncture with the facial bones, facilitate changes in the facial proportions and with these the conformation of the alveolar regions. Dr. Karl Gorjanovic-Kramberger insists that an intensive construction of the chin is possible only through a change in the angle of prognathism, and reduction in length and size of teeth. The change in the angle of prognathism throws the roots of the front teeth to the fore and bony tissue must be built up there to give them strength. With this change in the angle of the teeth the mental prominence becomes more pronounced and the construction of the chin is assured.³⁰ He alleges also, and with some show of reason, that the construction of the chin in modern man has been directly correlated with the arching of the prognathous mandible. Toldt, similarly, has attributed the formation of the chin to progressive changes accompanying the widening of the face and the arching of the mandible.³¹

The changes that have taken place with the assumption of the erect posture and the use of softer food, have been fairly uniform, and are seen in many portions of the skull. As the

²⁹ This has been ably demonstrated by Mr. Francis Knowles in his study of the correlation between the interorbital width and other measures and indices of the human skull. *Journ. of the Roy. Anth. Inst.*, 41 ('11), 318-49. Herman Weleker, in an article on *Die Zugehörigkeit eines Unterkiefers zu einem bestimmten Schädel*, etc. (*Archiv. f. Anthropologie* 27 ('01-'02), 37-106, has ably demonstrated the interdependence of the mandible and the skull. See also Aurel Von Török, *Über Variationen und Correlationen der Verhältnisse am Unterkiefer*; *Zeitsch. f. Ethnol.* 30 ('98), 125-182. The correlation is, of course, not absolute. Exceptions exist, but the reference to them as 'disharmonic' types is evidence of the rule.

³⁰ See the section on *Zur Bildung des Kinnes beim Homo primigenius*, in the author's *Der Diluviale Mensch von Krapina in Kroatien*, 171-176, (Wiesbaden, 1900).

³¹ *Corresp. Blatt. d. Deutsch Gesells. f. Anthrop.*, 35, ('04), 94; see *L'Anthropologie*, vol. 16, p. 583-584.

foramen magnum moves forward and the head becomes better balanced, less muscular pull is required to keep it in position. The mastoid muscles degenerate, pressure along the temporal bones decreases, the forehead emerges, and the head increases in breadth. The face, at the same time, broadens, because its foundation walls in the calvarium have begun to shift laterally. The alveolar processes must shift laterally also. This lateral spread of the palatal region arches the frontal portion of the alveolar region, which tends to be pointed or straight in the simia, and draws the incisors in, so that they no longer project, as hitherto. The face projects less, and with the straighter face the teeth, which in both simia and homo sapiens follow the facial angle, approximate the vertical; for the teeth must conform to the plane of the bony tissue in which they are set. Since the mandible is useful only as a correlative portion of the facial structure, it must conform to the changes effected in the skull. In doing so the chin becomes, of necessity, more and more prominent.

In the transition from the simian to the human type, we must take into account also the change that has come about in the articulation of the incisors. In the simia the incisors meet. This is not uncommon in palaeolithic man and in some of the more prognathous people. Among ourselves it still occurs, but only in a small percentage of cases. In the gorilla, indeed, the upper canines actually overlap the lower to a marked degree, so that the diastema on the lower alveolar region is much more marked than that on the upper. Already, then, the relative prominence of upper and lower incisors seems secured by this forward grasp of the upper canines. Man is standing proof of the triumph of the facial canines over the mandibular, for in homo sapiens all of the upper incisors, as well as the canines, easily overlap the lower, though sometimes the reverse occurs. It must, moreover, be borne in mind that the anatomical independence of the mandible, even where there is no physiological independence, allows the lower alveolar processes to accommodate themselves more rapidly to the new conditions, and we might expect that they would be more rapidly influenced by changes

in diet than would those of the palatal region. This would account for their more rapid retreat, while at the same time the enclosing upper incisors help by means of pressure from without.

We do, as a matter of fact, find that the changes in the mandible have gone further than those in the facial portions. In primitive man the alveolar processes are still at the outer margin of the lower facial region. But not so in the jaw of civilized man with prominent chin, where the alveolar processes lie behind the underlying heavy bony tissue, so that the teeth no longer conform to the plane of the bony tissue in which they are embedded. We do not find such considerable changes in the facial portion as in the mandibular.

We conclude, then, that no one factor should be singled out and given the credit for having evolved the chin, but that many forces have contributed to this result. Widening of the dental arcade gives more play for the tongue, whether for shifting food or consonants. It makes a better masticator, and perhaps, a better linguist. The interrelation may be close. Which is cause and which effect may be difficult to determine, for we have, not one, but many, parallel or interrelated developments converging in that peculiar human feature, the chin, which muscular forces, both within and without, have helped to design.

NOTES ON TWO CASES OF ANOMALOUS RIGHT SUBCLAVIAN ARTERY

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Cases of anomalous right subclavian have been reported occasionally, but their rarity and interest from an embryological point of view, besides their possible clinical relations, warrant their addition to the anatomical literature. Holzapfel ('99) collected two hundred cases of abnormal right subclavian artery including four of his own, and discussed them from anatomical, developmental, and practical stand-points. He gave the frequency as one case to one hundred sixty-seven cadavers, or .6 per cent. Cobey ('14) gives the results of an investigation for the Anatomical Society of Great Britain and Ireland as five to five hundred, or 1 per cent. The cases here reported are two to two hundred thirty-seven cadavers, or 0.8 per cent. Bean ('04) reported two cases of his own and six others in the literature besides Holzapfel's cases. Cobey and Bevier ('15) have reported each one case.

Case 1. The subject is a white male, age sixty years. The arcus aortae gives off three branches, the truncus bicaroticus, arteria subclavia sinistra, and arteria subclavia dextra, the anomalous branch. It extends in a gentle curve upwards, backwards, and to the right from the level of the lower border of the first left costal cartilage to the left side of the body of the third thoracic vertebra. This abnormal course of the arcus aortae is produced by the rotation of the base of the heart towards the left. The aorta ascendens, therefore, lies ventrad and to the left of the aorta descendens, both lying to the left of the vertebral column. The truncus bicaroticus is flattened ventrodorsally, and, of course, lies to the left of the trachea and the midline. The A. subclavia sinistra is the second branch. The third branch is the anomalous A. subclavia dextra arising from the right side of the aorta descendens at its commencement. It crosses the vertebral column behind the oesophagus and trachea, diagonally cephalad and to the right, lying on the body of the third thoracic vertebra. It is considerably dilated up to the point where it emerges from behind the oesophagus. The great veins are normal. The trachea is normal. The oesophagus shows an interesting abnormality by making a detour to the right for a distance of 10 mm. where the anomalous A. subclavia dextra emerges dorsad to it. The right vagus preserves its usual course in the thorax. The N. recurrens is absent, the cardiac branch

of the vagus, usually arising from the recurrens, being supplied directly from the vagus trunk. Three or four twigs, taking the place of the recurrens, pass from the nerve trunk directly to the larynx. The left vagus is normal in its course and branches. The N. recurrens, however, passes across the ventral aspect of the anomalous subclavian. The ductus thoracicus is normal in its course. The phrenic nerves are normal.

Case 2. The subject is a white male of advanced years. The arrangement of the arcus aortae and its branches is similar to that described in Case 1. However, the truncus bicaroticus is cylindrical, and the abnormal A. subclavia dextra is not dilated.

In Holzapfel's cases 33 were dilated out of 51 in which that feature of the abnormal artery was discussed, or 64 per cent. One of the cases collected by Bean was dilated. The clinical effects of a dilated abnormal artery are to be taken into account, therefore. Holzapfel discusses this point and concludes that only by an aneurysmal enlargement of the artery may dysphagia lusoria be considered.

Surgically the abnormal course of the artery is important in attempts at ligation; in one instance reported it offered a decided difficulty. In surgical conditions involving the oesophagus or in operations on the thorax its presence may be of considerable consequence. The internist should be interested in the possibility of its causing unequal radial pulsations. Holzapfel believes it is not the anatomical cause of left-handedness, although it is to be noted that the two conditions exist in several cases. Cobey suggests the possibility of the abnormal artery producing symptoms similar to those of cervical rib, with resulting trophic changes in the extremity.

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日本解剖學者及動物學者諸君

謹啓 現下歐洲ニ於ケル不幸ナル戰禍ハ各方面ニ少ナカラヌ影響ヲ及ボシ候ガ就中科學的研究ハ多大ナル阻碍ヲ蒙リ歐洲ニ於ケル解剖學及動物學ニ關スル幾多ノ雜誌ハ或ハ發行中止ノ己ムナキニ至レルモノアリ然ラザルモノモ之ヲ米國ニ於テ手ニ入ルコト甚ダ困難ナル狀態ニ有之申候 就テハ

形態學雜誌、比較神經學雜誌、解剖學雜誌、解剖學彙報、實驗動物學雜誌五雜誌ノ編輯局ハ此時局ニ際シテ及ブ限り右學術ノ研究ニ對シ補助ト獎勵ト相與ヘシ希望ヲ以テ世界各國研究所ノ業績ニ對シ廣ク出版上ノ便宜ヲ斗ルコトニ相成申候 即チ解剖學及動物學上ノ諸論文ハ其編輯當局ノ認許ヲ得次第迅速ニ英、佛、獨、伊及西班牙語ノ何レニテモ登載可仕候 從來上記ノ五雜誌ハ生物學ニ關スル米國研究家ノ業績ノ大多數ヲ網羅シ且世界各國ニ最モ廣ク汎布致サル有カナル雜誌ニテ孰レモ米國貴府ウィスター研究所カ斯學推輓ノ目的ニテ出版致シ居ルモノニ有之其編輯局ハ米國及加奈太ノ重ナル代表的研究家ノ殆ド凡テヲ包含致居候 就テハ今回右編輯局ハ日本帝國ニ於ケル解剖學及動物學者ノ御便宜ノ爲メ其業績ヲ右五雜誌ニ於テ發表セラレンコトヲ切望致候 而テ之ニ依テ將來日米兩國研究者間ノ親交ヲ幫助スルコトヲ得バ至幸實ニ之ニ過ギスト存候 尚出版ニ關スル凡テノ御照會ハ何卒尤記ヘ宛テ御文通被成下度願上候 敬具

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ウィスター解剖學及生物學研究所 所長 ドクトルミルトン・ジエ、グリーンマン

A causa de las lamentables condiciones por que atraviesa Europa, en algunos de los países más importantes del mundo se han interrumpido seriamente los trabajos de carácter científico, y una gran cantidad de revistas anatómicas y zoológicas han suspendido su publicación, mientras que otras se han vuelto más o menos inaccesibles para los investigadores, y con especialidad para los investigadores que trabajan en los laboratorios americanos.

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Para mayores informes dirigirse a

DR. MILTON J. GREENMAN, Director de
The Wistar Institute of Anatomy and Biology
Philadelphia, Pa., U. S. A.

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THE LYMPHATICS SYSTEM OF THE GUINEA-PIG

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NINETEEN FIGURES

INTRODUCTION

This investigation of the lymphatic system of the adult male guinea-pig was entirely macroscopic. Owing to the technical difficulties the lymphatics of certain organs of the body such as the stomach were not injected successfully. Yet, in the more general sense, apart from the minor details, the fundamental plan of the system of the guinea-pig was revealed.

The literature on the anatomy of this animal is very limited but the anatomy of the bones and the muscles by Alezais, '98-'02 has materially assisted me in this study.

The method of investigation was as follows: The animal was killed with ether and while it was still warm, injections were made by means of the ordinary hypodermic syringe provided with a fine glass cannula. The animal injected was then carefully dissected in the fresh or after temporary preservation. The needle must be of very fine caliber, indeed, in order to obtain a successful injection of the lymphatics of the guinea pig. The finest hypodermic needles obtainable in the market are too large, for their use usually causes a large area of the extravasation without filling any lymphatic vessels. Such a needle was, however, improved by fusing on to it a fine glass cannula, the tip of which was drawn out into a short but very delicate capillary tube. Among the various injection masses that were tested, Gerota's Prussian blue and diluted India ink gave the most satisfactory results.

This investigation is based upon experiments on about 30 male guinea-pigs, which after the injection, were carefully dissected with the aid of a watch-maker's lens.

Injection of the cutaneous lymphatics offered no little difficulty when I attempted to work from the outer surface of the skin, for the needle either penetrated too deep or too superficially. This difficulty was overcome by reflecting a small flap of skin by a crescentric incision and inserting the needle from the under surface. However injection must be made very slowly and under low pressure.

Working with the mesenteric nodes, it was found that when any of them were forcibly distended with injection fluid in order to inject the lymphatics backward to the loop of the intestine, the veins received retrograde injection instead as mentioned previously by Meyer, '14.

For the purpose of description the body is divided into the following regions: the head and neck, the upper extremity, the lower extremity, the thorax and the abdomen. The lymph-glands of the entire system are first described with reference to their average size, general shape, location and their afferent and efferent vessels. This is followed by the consideration of the lymphatic vessels of each region. The names of the glands are adopted, as far as possible, from the corresponding glands of the human lymphatics, but there are a few glands, which because of their closer similarity to those of cattle, are named after the work of Baum, '12. However, since some glands are situated quite differently in the guinea-pig from corresponding glands in man and other animals, it seemed better to adopt new names than to rigidly adhere to a given nomenclature. These glands were the following:

A. Infra-mandibular node. From the efferent and afferent vessels, the node corresponds to the submental node. It lies behind the mandible and the term inframandibular node seems quite appropriate.

B. Dorsal and ventral pre-scapular node. These nodes correspond to the deep cervical nodes but since they lie at the level of the cephalic border of the scapula the term pre-scapular seems the more appropriate.

C. Retro-scapular node. This belongs to the group of axillary nodes but lies wholly outside of the axillary space so that the term axillary node could only express a rough correspondence. Since it is more closely in relation with the caudal border of the scapula, the term retro-scapular seemed quite justifiable.

D. Abdomino-inguinal and inguinal nodes. These nodes correspond to superficial and deep inguinal nodes respectively but their relation is so disturbed by the peculiar position of the hind limb that what might be the superficial inguinal node is pushed out of place and lies on the abdominal wall under cover of the tensor fascia lata, so that the term abdomino-inguinal is suggestive both of position and correspondence.

The term inguinal node instead of deep inguinal is used because there is no superficial inguinal node. Consequently the adjectives 'superficial and deep' are superfluous and the term inguinal is employed to indicate a gland homologous with the deep inguinal node.¹

1. THE LYMPH GLANDS

Lymphoglandula parotidea (figs. 1, 15.) Position and size. The gland is found caudal to the base of the auricle, on the dorso-caudal border of masseter muscle and on the external maxillary vein. It is covered by the cephalic portion of the platysma and skin and is imbedded in the parotid gland. Unless injected, it is very difficult to recognize it. It is flat and ovoid in shape, measuring 4 by 3 by 2 mm.

Afferent vessels. The chief afferents of the auricle terminate in this node.

Efferent vessels. One or two vessels from the node follow the external maxillary vein into the substance of the parotid gland and terminate in the submaxillary node which is placed at the junction of the external and internal maxillary vein and covered partly by the submaxillary salivary gland.

Lymphoglandula inframandibularis (figs. 1, 2) This gland is located on the ventral surface of the anterior belly of the digastric muscle in close proximity to that on the opposite side. These nodes are usually placed transversely and one of them may be found quite behind (caudal to) the other. They are covered by the platysma and the skin. They are round and flat, 5 to 6 mm. in diameter and 2 mm. in thickness.

¹ A supernumerary adrenal body was often found near the renal vessels. This was identified histologically.

It is not, however, infrequent to find one of them considerably larger and more elongated and measuring 9 mm. in length.

Afferent vessels. The lymphatics of the lower lip and those of the anterior two-thirds of the tongue terminate in this node.

Efferent vessels. One or two vessels pass transversely between the sterno-hyoid and digastric muscles where they join with the collectors from the posterior part of the tongue. These conjoined vessels are directed caudal on the superficial surfaces of the sterno-hyoid and scalenus anterior and terminate in the deep cervical glands.

Lymphoglandula submaxillaris (figs. 1, 2, 15) Situation and size. This node which is entirely hidden by the parotid and submaxillary

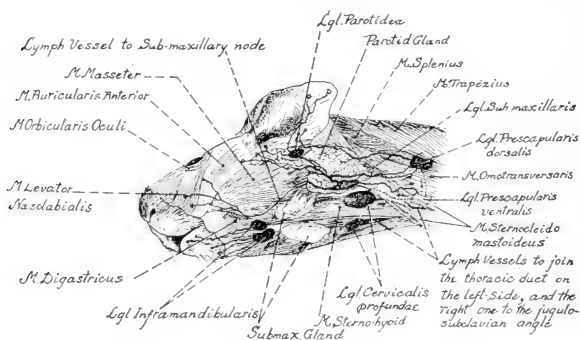


Fig. 1 Superficial lymphatics of the neck

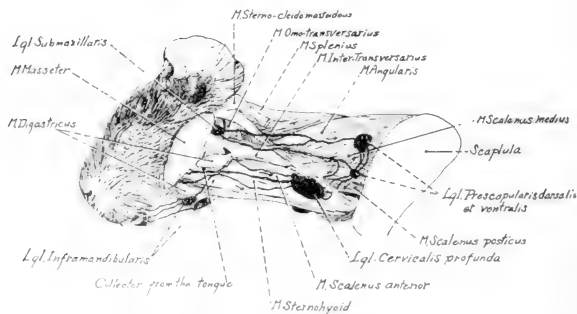


Fig. 2 Deep lymphatics of the neck

salivary glands lies at the junction of external and internal maxillary veins and is covered by the platysma. It is an irregularly shaped gland measuring about 4 mm. in diameter and 2 mm. in thickness. It is often represented by two glands; one medial to the other; but in very close relation to each other and separated only by the small portion of the submaxillary salivary gland and a part of the veins. When double these glands are connected with a number of short anastomosing lymph vessels.

Afferent vessels. The vessels from a small portion of the auricle, from the frontal cutaneous area around the eye-lids, and efferents of the parotid node terminate in this node.

Efferent vessels. Two or three vessels from this node follow the external jugular vein. At the lower border of the sternocleidomastoids, the vessels take three different directions. One passes over the dorsal and the other over the ventral and dorsal prescapular nodes. Another vessel passes directly into the deep cervical nodes.

Lymphoglandula prescapularis dorsalis (figs. 1, 2, 15). This node lies on the angularis muscle cephalad to the scapula and is covered by the omotransversarius and trapesius muscles. It is ovoid in shape, measuring 5 to 7 mm. long, 4 mm. wide and 3 mm. thick.

Practically all the cutaneous lymphatics of the neck and those from the skin covering the cephalic portion of the scapula and the efferents from the parotid and submaxillary nodes terminate in this node.

Two or three vessels are directed ventrally to end in the ventral prescapular node.

Lymphoglandula prescapularis ventralis (figs. 1, 2). This gland is rarely absent. It lies at the root of the neck on the scalenus medius, and is covered by the clavicular portion of the sternocleidomastoid and platysma muscles. It is round and flat, measuring 4 mm. in diameter and 2 mm. in thickness.

Two or three vessels from the dorsal prescapular and one or two from the submaxillary nodes terminate in this node.

Usually two vessels pass ventro-cephalad under the sternocleidomastoid muscles to the deep cervical nodes.

Lymphoglandula cervicalis profunda (figs. 1, 2). This gland lies at the bottom of the V-shaped space formed by the sternocleidomastoid laterally, and the sternohyoid medially and is in contact with the trachea and anterior scalenus muscles lying in close relation to the internal jugular vein and the carotid artery. It is the largest node in the region of the head and neck. It is ovoid in shape and flattened, being 8 to 11 mm. long, 6 mm. wide and 3 mm. thick.

All lymphatics from the head and neck finally terminate in this node. It receives the collectors from the tongue and the efferent vessels from the submandibular, submaxillary and ventral prescapular nodes.

The efferent vessel from this node is the largest lymph vessel in the neck. It is 12 to 15 mm. long, distinctly sacculated and closely follows the internal jugular vein, lying between the sternocleidomastoid

and the anterior scalenus muscles. At the root of the neck it terminates on the left side at the cervical bend of the thoracic duct and on the right side, at the junction of the internal and external jugular veins.

2. The lymphglands of the anterior extremity

Lymphoglandula retro-scapularis (figs. 4, 5). This node is located on the outer surface of the latissimus dorsi at the angle formed by the caudal border of infraspinatus and the dorsal border of the triceps brachialis. It is imbedded in a pad of fat and covered by the panniculus carnosus and the skin. It is ovoid in shape and flat, 9 mm. long, 4 mm. wide and 2 mm. thick.

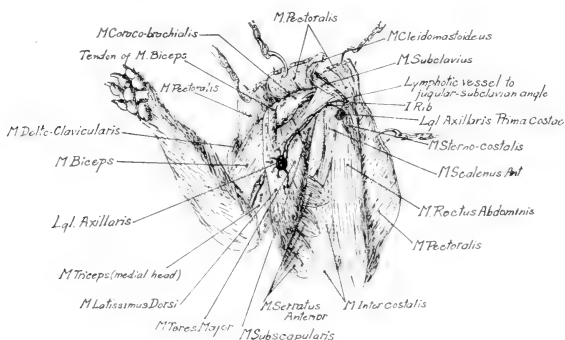


Fig. 3 Lymphatics of the axilla

The lymphatics of the outer surface of the anterior limb, and the dorsal and lateral cutaneous lymphatics of the thorax terminate in this node.

Two or five vessels from this gland penetrate the latissimus dorsi and terminate in the lymphoglandula axillaris.

Lymphoglandula axillaris (figs. 3, 17). This gland lies on the medial surface of the teres major at the angle formed by the axillary artery and the tendinous insertion of the latissimus dorsi. Medially it is in relation with the scalenus anterior. It is a circular node 5 to 7 mm. in diameter and 2 mm. thick.

It receives efferent vessels from the retro-scapular node and the inner cutaneous lymphatics from the pectoral region also terminate in it.

The large single trunk which follows the axillary artery is interrupted by the lymphoglandula axillaris prima costae. However, it is not infrequent to find this trunk dividing near the latter node into two

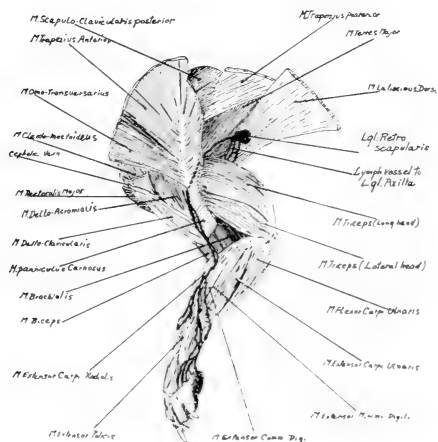


Fig. 4 Lymphatics of the fore limb

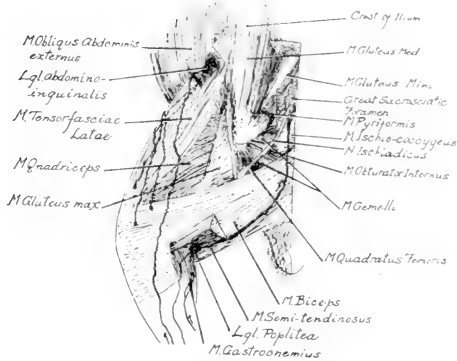


Fig. 5 Lymphatics of the thigh

vessels, one of which terminates in this node and the other at the junction of the external jugular and subclavian veins.

Lymphoglandula axillaris prima costae (figs. 3, 17). This node is found on the outer surface of the first rib, at the termination of the axillary blood vessels, being covered by the pectoral muscles. It is circular and about 5 mm. in diameter and 2 mm. in thickness.

The efferent vessels of the lymphoglandula retro-scapularis and very often some efferents of the lymphoglandulae tracheobronchiales and lymphoglandula axillaris end in this node.

A large single trunk leaves the node and terminates at the junction of the subclavian and external jugular veins.

3. *Lymphatics of the lower extremity*

Lymphoglandula poplitea (figs. 5, 7, 15). The node lies imbedded in the popliteal fat, in the triangular space bounded by the biceps femoris, semitendinosus and gastrocnemius muscles. It is spherical in form and about 2 to 3 mm. in diameter.

The lymphatics of the lateral surface and dorsum of the foot terminate in it.

The majority of the efferent vessels follow the popliteal blood vessels where they join with the deep lymphatic vessels of the hind limb. However, there is one other efferent vessel which passes along the postero-caudal border of biceps the femoris. Near the origin of the latter it crosses the outer surface of the muscle and soon passes under the gluteus maximus to accompany the ischiadic nerve with which it enters the pelvic cavity through the foramen ischiadicum majus. In the pelvis it lies close to the median line on the anterior surface of sacrum and finally terminates in the hypogastric node.

Lymphoglandulae abdomino-inguinales (figs. 5, 6, 7, 15). These glands are found along the course of the superficial circumflex iliac vessels between the obliquus abdominis externus and tensor fasciae lata muscle. This group consists of 2 to 4 nodes of varying size, the largest of which reaches about 7 mm. in diameter.

All cutaneous lymphatics of the abdomen and the hip, the lymphatics of the outer and inner surfaces of the thigh, those from the scrotum and from a small portion of the lateral part of the hind leg terminate in this node.

The majority of the efferent vessels accompany the superficial circumflex iliac vessels and terminate in the inguinal nodes but a small vessel passes through the femoral ring to end in the iliac node.

Lymphoglandulae inguinales (figs. 6, 7). This pair of nodes is found on the femoral vessels, close to the femoral opening and is composed of a medial and a lateral node. The medial node is irregular in shape measuring about 5 mm. in diameter and 2 mm. in thickness. The lateral node is the smaller of the two. It is circular and measures about 3 to 4 mm. in length and 1 mm. in thickness.

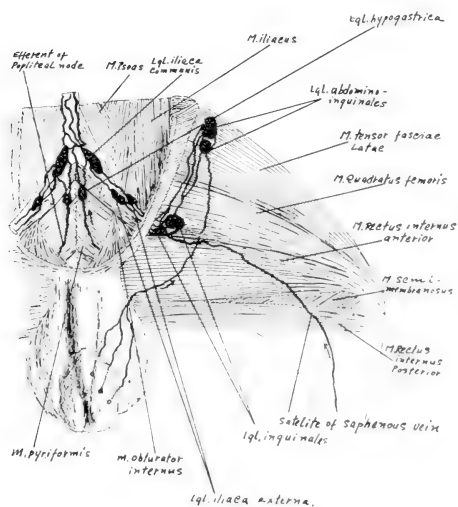


Fig. 6 Lymphatics of the femoral and pelvic regions

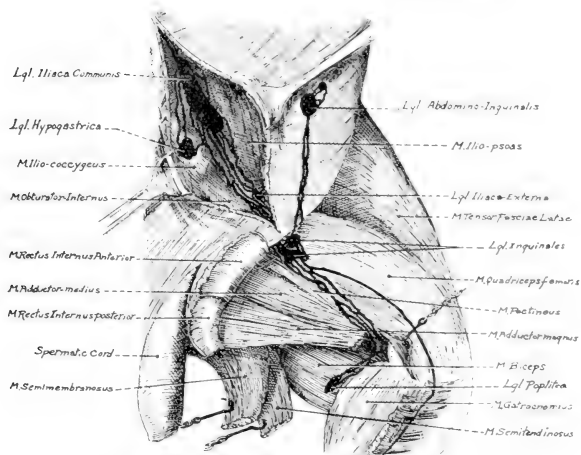


Fig. 7 Lymphatics of the abdominal and femoral regions

The efferents from the popliteal nodes, the deep lymphatics of the hind limb and those accompanying the saphenous artery terminate in the medial, while the efferent vessels of the abdomino-inguinal nodes terminate in the lateral node. These two nodes are in communication with each other.

A number of vessels pass through the inguinal canal and terminate either in the external iliac or in the common iliac nodes.

4. The Lymph glands of the abdomen

Lymphoglandula iliaca externa (figs. 6, 7). This is a very inconstant gland located when present on the external iliac vessels. It is quite round and 3 mm. in diameter and 2 mm. in thickness. It is interpolated in the course of the efferent vessels coming from the inguinal nodes but sometimes also receives afferents from the bladder.

Lymphoglandula hypogastrica (figs. 6, 7, 10, 16). This is a small but constant gland lying in the pelvic cavity at the origin of the hypogastric artery. It is a small circular gland 2 mm. in diameter and 1 mm. in thickness.

The lymphatics of the bladder and the seminal vesicle and the efferent vessel from the popliteal node terminate in it.

An inconstant number of efferents cross the common iliac vessels to terminate in the common iliac node while certain other vessels pass toward the abdominal aorta, contributing to the formation of the lymphatic plexus around this vessel and the inferior vena cava.

Lymphoglandula iliaca communis (figs. 6, 7, 10, 16). This node is placed at the angle of abdominal aorta and the common iliac artery. Indeed it is not infrequent to find it lying entirely on the outer side of the common iliac vessels. It is fusiform in shape, 7 mm. long, 3 mm. wide and 2 mm. thick.

The entire lymph stream of the lower extremity and from the pelvic cavity reaches it. Vessels from the bladder and from the pelvis together with the efferent vessels of the inguinal, the external iliac and the hypogastric nodes terminate here.

As soon as the efferent vessels from this node reach the side of the great abdominal vessels, they form a very rich plexus which entirely surrounds these vessels. Their final destination is the cisterna chyli, but before reaching it they are interrupted by small 'Schaltdrüsen' scattered in the course of the plexus.

Lymphoglandulae mesentericae intestinales (fig. 9). A large number of lymph nodes of varying size are found between the two layers of the mesentery. Among these is one large node which lies on the main trunk of the mesenteric vessels. This node receives the entire lymph stream of the intestine. The rest of the nodes are scattered on the peripheral branches of the same vessels, constituting glands for different segments of the intestine. These latter glands are quite variable. Hence the description of a single type of arrangement can not be entirely satisfactory. Nevertheless, there is a certain prevailing arrangement which is considered here as typical.

The following glands constitute the lymphoglandulae mesentericae intestinales:

1. The lymphoglandula colicae descenditis.
2. The lymphoglandula colicae transversalis.
3. The lymphoglandula colicae ascenditis.
4. The lymphoglandula intestini tenuis.
5. Lymphoglandula ilio-coecalis.
6. Lymphoglandula coecalis.
7. Lymphoglandula mesentericae communis.

1. Lymphoglandula colicae descenditis. This node is found close to the middle of the descending colon, imbedded in a small quantity of fat. It is somewhat round in shape, 2 mm. in diameter and 1 mm. thick.

Lymph from the descending colon drains into this node.

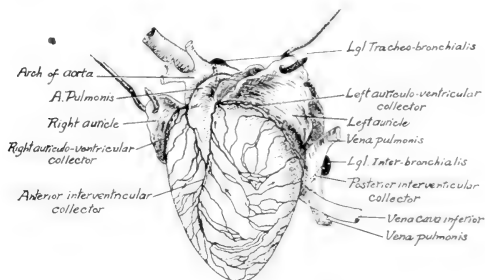


Fig. 8 Lymphatics of the heart

One small vessel from it follows the inferior mesenteric artery and at the origin of the latter joins with the lymph plexus surrounding the abdominal aorta.

2. The lymphoglandula colicae transversalis is located close to the middle of the transverse colon. It is round in shape, 5 mm. in diameter and 2 mm. in thickness.

The lymphatics of the transverse colon end in it and the efferent vessels usually reach the common mesenteric node.

3. Lymphoglandula colicae ascenditis. This is found close to where the ascending joins the transverse colon. It is 4 mm. long, 3 mm. wide, 3 mm. thick and is not rarely accompanied by other very small glands close to it.

The majority of the afferent vessels draining into this node are from the ascending colon but a few vessels from the transverse colon also end here.

Lymph from it is received into the common mesenteric nodes.

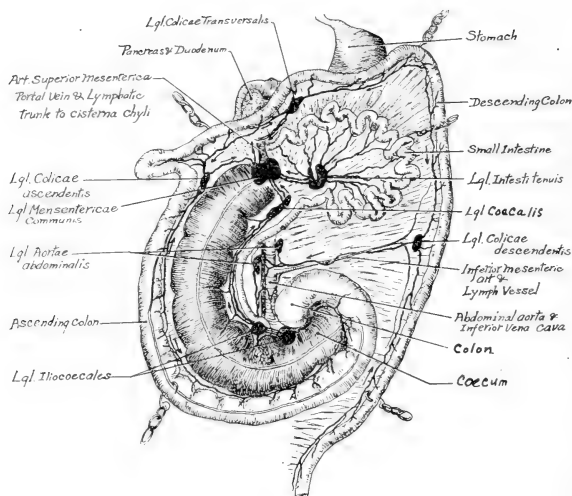


Fig. 9 Lymphatics of the intestine

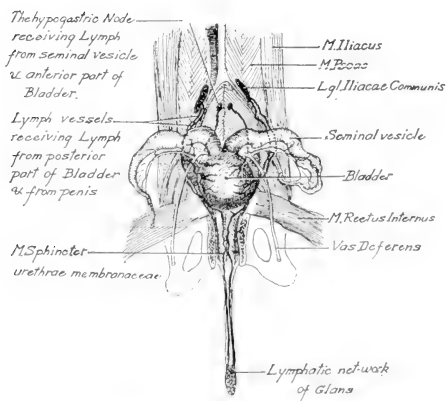


Fig. 10 Lymphatics of the genito-urinary organs

4. *Lymphoglandula intestini tenuis*. The gland of the small intestine lies near the center of the mesentery of the small intestine. It is an irregularly shaped node about 10 mm. in diameter and 2 to 3 mm. in thickness.

A large number of afferents from the small intestine converge toward it.

Two or three vessels from this gland terminate in the common mesenteric glands.

5. *Lymphoglandula ilio-coecalis*. A glandular mass constituting the ilio-coecal node is located around the spot where the ileum and caecum meet. This composite mass may be grouped into one node measuring about 10 mm. in diameter and 4 mm. in thickness but it is more often divided into two unequal masses.

The lymph vessels from a small portion of the ascending colon and from the caudal half of the caecum terminate in it.

Two or three vessels from this node traverse the mesentery stretched between the caecum and ileum to reach the coecal node.

6. *Lymphoglandula coecalis*. This node lies in the mesentery between the ileum and the caecum close to the common mesenteric node and the gland of the small intestine. Sometimes it fuses with the gland of the small intestine. It is somewhat elongated, measuring 7 to 10 mm. long, 3 mm. wide and 2 mm. thick.

Many of the coecal lymphatics terminate in this node, as do also the efferents of the ilio-coecal node.

Two or three large vessels from it end in the common mesenteric node.

7. *Lymphoglandula mesentericae communis*. This is the most important gland of the intestine. It lies on the trunks of the mesenteric blood vessels just before these cross the duodenum and is in close relation with the lymph gland which drains the small intestine and with the transverse colon and the head of the pancreas. It is often divided by the pancreas, one portion lying anterior and the other posterior to it. Behind this node lies the apex of the caecum and a portion of the duodenum. It is irregular in shape, about 8 mm. long and 3 mm. thick. In a number of cases it is not a separate node but forms one large mass with the glands of the small intestine and the appendix. However, separation into three distinct nodes is more common.

With the exception of the lymphatics from the descending colon, the entire lymph from the intestine drains into this node. Its afferent vessels come from the coecal, ilio-coecal and intestinal nodes.

The single large distinctly sacculated truncus lymphaticus intestinalis which leaves this node closely follows the course of the superior mesenteric artery and empties into the cisterna chyli. Often a small vessel is sent from this trunk to the retropancreatic node.

Lymphoglandula retropancreatica (fig. 11). This gland is found behind the head of the pancreas on the superior mesenteric vein near the foramen epiploicum. It is a pear-shaped or somewhat ovoid node about 8 mm. long.

A number of vessels from the liver and a small vessel from the common mesenteric node end in it.

One to four vessels are given off from this node and either join with the truncus intestinalis or follow the superior mesenteric artery and terminate in the cisterna chyli.

Lymphoglandulae aortae abdominalis (fig. 16) There are a large number of glands around the abdominal aorta and the inferior vena cava. These glands are divided into three groups: the inferior, middle and the superior. But it must be remarked that these divisions are absolutely artificial, for the nodes form a continuous chain without any distinct separation. Hence it is difficult to group separately. Some lymphatic vessels go to certain of these nodes but the territory drained is not distinct.

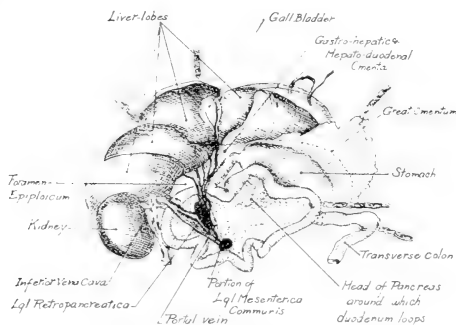


Fig. 11 Lymphatics of the liver

1. The lymphoglandulae aortae abdominalis inferiores are composed of three to six glands about the size of a pin's head which are grouped around the lower half of the great abdominal vessels, in meshes of the lymphatic plexus. These nodes are nothing more than 'Schalt-drüsen' interrupting the lymph from the hypogastric and the common iliac nodes and from the descending colon on its way to the cisterna chyli.

2. Lymphoglandulae aortae abdominalis mediales. This group of nodes is found around the abdominal aorta, the renal vessels and the superior mesenteric and spermatic arteries. The glands of this group are much larger than those of the inferior mesenteric arteries. They are usually ovoid in shape and the largest one may be as big as 5 mm. but very many small nodes may be found among them.

Since these glands lie in a plexus of lymphatic vessels, some of them are only interrupting nodes while others receive definite afferents from the kidneys and the testicles.

The efferent vessels from them terminate in the cisterna chyli which lies behind the aorta, between the pillars of the diaphragm.

3. *Lymphoglandula aortae abdominalis superior*. This node lies against the lateral surface of the pillars of the diaphragm. It is ovoid or round measuring about 4 mm. across.

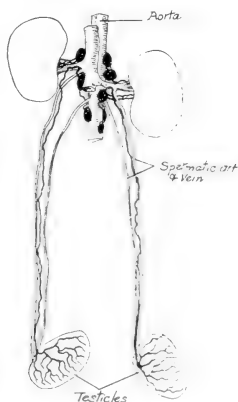


Fig. 12 Lymphatics of the testicle and kidney

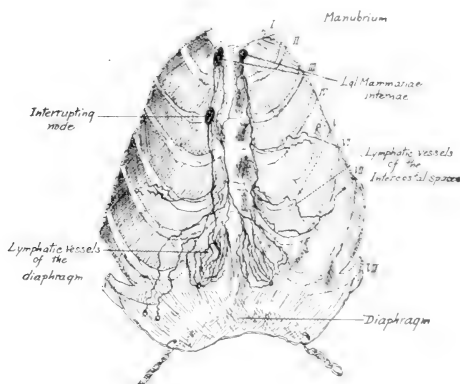


Fig. 13 Lymphatics of the diaphragm

Some afferents from the kidney and others from the abdominal plexus, which do not empty into the cisterna chyli at this level join it. Usually one of these vessels either penetrates the pillar of the diaphragm or passes behind the arcuate ligament and joins the cisterna chyli at once.

5. The lymph glands of the thorax

Lymphoglandulae arteriae mammariae internae (figs. 13, 17). One node is located internal to the first rib in company with the internal mammary vessels. Cranially it is in relation with the origin of the sterno-hyoid and sterno-thyroid muscles and dorsally it is in relation with the innominate vein and the arch of the aorta. It is a small, somewhat round gland, measuring about 3 mm. in diameter.

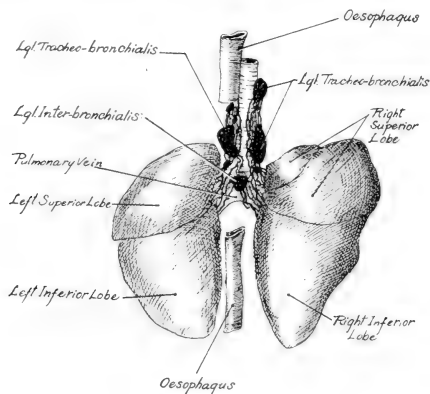


Fig. 14 Lymphatics of the lung

Lymphatics from the diaphragm and from the intercostal spaces terminate in this gland and it is usual to find that some vessels from the tracheo-bronchial nodes join it also.

The efferent vessels from the nodes of both sides form complicated plexuses on the innominate vein. The plexus on the left side joins with the ductus thoracicus and on the right with the cervical and the subclavian lymph trunks just before their termination in the vein.

Lymphoglandula inter-bronchialis (fig. 14). A single node is located at the bifurcation of the trachea between the roots of the bronchi. Ventrally it is in relation with the pulmonary vein and dorsally with the oesophagus. It is a somewhat spherical node measuring about 3 mm. in diameter.

The lymphatics of the lung and heart send some of their lymph vessels to it and the efferent vessels terminate in the tracheo-bronchial nodes of both sides.

Lymphoglandula tracheo-bronchiales (figs. 14, 17). These nodes lie in the angles between the trachea and bronchi on the right and left side respectively. They are elongated thick nodes 10 mm. long, 4 mm. wide and 3 mm. thick and are often separated only by the azygos vein which crosses the united nodes.

They receive afferents from the lung and the efferents from the bronchial node. The lymph vessels joining the glands of both sides of the trachea are often found to anastomose with each other and also with a number of anastomatic vessels which surround the trachea.

The efferent vessels from this node terminate in three possible places: in nodes along the internal mammary artery, in the lymphoglandulae axillaris prima costae or in the plexus in front of the innominate vein.

II. THE LYMPH VESSELS

6. *The lymphatic vessels of the head and neck*

1. *The lymphatics of the ear* (figs. 1, 15). The lymphatics of the ear are best injected on the medial side. The network appears near the margin and from this plexus appear a number of vessels which join with each other as they approach the root of the ear. The majority of these radicles pass to the parotid node but a few pass directly to the submaxillary lymph node.

2. *The lymphatics of the face* (figs. 1, 15). A large number of very fine vessels arise in the frontal region from the eye-lids, the side of the nose and from the upper lip. They all run toward the lateral surface of the neck, penetrate the platysma and come to lie between it and the masseter muscles. The larger collectors, however, follow the internal maxillary and branches of the external maxillary vein. All these vessels converge to the submaxillary lymph nodes. The lymphatics of the lower lip terminate in the inframandibular node.

3. *The cutaneous lymphatics of the neck* (fig. 15). All the cutaneous lymphatics of the neck which are subcutaneous at first penetrate the platysma. They then pass either through the dorsal or ventral border of the omotransversarius and terminate in the dorsal prescapular node.

4. *The lymphatics of the fore-limb* (figs. 3, 4, 15). When injections are made into the pad of the foot of the anterior limb, several vessels are seen to pass centrally in various directions. Some pass between the digits, others curve around the radial and ulnar borders of the foot to appear on the dorsum and still others pass to the flexor surface of the forearm. The latter have an entirely different path and termination from the former. Those that appear on the dorsum of the foot pass to the extensor surface of the forearm where they join to form one

vessel which accompanies the cephalic vein to the middle of the brachium. Here it leaves the vein and penetrates the panniculus carnosus, terminating at once in the retroscapular node.

Those vessels which appear on the flexor surface of the forearm continue their course as far as the distal third of the brachium where they meet the brachial artery. Here they join with the deep lymphatic vessels which accompany this artery and end in the axillary node.

5. *The lymphatics of the hind limb* (figs. 5, 6, 7, 15). A number of lymphatic vessels which arise from the medial and lateral margins of the foot arrange themselves on the lower part of the leg into medial and lateral collectors. The medial collector follows the saphenous vein very closely and terminates in the inguinal nodes. The lateral collectors pass along the outer surface of the leg and the majority terminate in the popliteal node. However, one or two vessels continue their course over the thigh and end in the abdomino-inguinal node. Indeed, the entire cutaneous lymphatics of the thigh converge to the latter nodes.

The deep lymphatics of the hind limb were not successfully injected except those along the femoral artery in the region of the thigh. If the pressure is relieved along the femoral artery by cutting away most of the adductor muscles, injections into the popliteal node always fill the deep lymphatic vessels which form a plexus around the artery and terminate in the deep inguinal nodes.

7. *The lymphatics of the abdomen*

1. *Cutaneous lymphatics of the abdomen* (fig. 15). Fine lymphatic vessels which arise from the skin of the abdominal wall soon penetrate the panniculus carnosus and join with one another as they converge to the abdomino-inguinal nodes. Separation of this area from that of the thorax is not sharply marked off for one merges into the other. Hence, an injection in the wide common zone which constitutes the boundary between them, may be seen to travel either to the external retroscapular or to the abdomino-inguinal node.

2. *The cutaneous lymphatics of the perineo-pudendal area* (fig. 6). From the network of origin on the skin in the perineal and pudendal regions arise one or two collectors which pass between the thigh and the abdomen to end in the abdomino-inguinal node.

3. *The lymphatics of the bladder* (fig. 10). A fine lymphatic network from which a number of collectors leave is found on the bladder. These collectors are divided into anterior and posterior collecting trunks from the anterior and posterior surfaces of the bladder respectively. The anterior gain the brim of the pelvis and usually join the common iliac nodes while the posterior collectors run to the floor of the pelvis to reach the hypogastric node.

4. *The lymphatics of the seminal vesicles* (fig. 10). From the very fine network of origin on the surface of the seminal vesicle arise a number of collectors which join with each other at the free border of

the mesentery of the vesicle to form one common vessel which at the side of the bladder joins with the collectors from the posterior surface of the latter. This conjoined vessel runs over the floor of the pelvis to reach the hypogastric node.

5. *The lymphatics of the penis* (fig. 10). The lymphatics of the prepuce and glans form two collectors which run along the dorsum of the penis. They pass under the symphysis of the pubis, over the sphincter urethrae muscle. Close to the bladder they diverge and after reaching the pelvic brim follow the external iliac vessels and terminate in the common iliac nodes.

6. *The lymphatics of the testicle* (fig. 12). When injections are made into the substance of the testicle a large number of fine vessels appear on the surface. These vessels converge to the point where the spermatic vein leaves the testicle where they form common collectors. These collectors, two or three in number, follow the spermatic vessels. The artery and vein are closely interwoven by these lymphatic vessels in their entire course. Near the kidney, the lymphatic vessels leave the artery, but follow the vein and after reaching the wall of the renal vein terminate in some of the nodes of the middle abdomino-aortic group.

7. *The lymphatics of the kidney* (fig. 12). The deep lymphatics of the kidney are easily injected by simply forcing the injection mass into the kidney substance. A number of lymphatic vessels appear immediately at the hilum. These surround the renal blood vessels and terminate in the nodes in the middle abdominal aortic group.

8. *The lymphatics of the intestine* (fig. 9). Working out the lymphatics of the intestine is a difficult task in a small animal. Owing to the delicacy of the intestinal wall, direct injection with the needle was unsuccessful. In the caecum and a small portion of the large intestine, however, injections made on the teniae were quite successful in demonstrating the fine plexus on the walls. To inject the lymphatics of the other portion of the intestine, the following method was employed: (1) the intestine was cut into pieces about 8 cm. long, the mesentery being kept intact as much as possible. The contents were washed out thoroughly and a small quantity of rather coarse sand was introduced. This was rubbed against the intestinal wall by rolling the intestine between the fingers, so as to injure the interior of the intestine. The intestine was then flushed again and every drop of water pressed out. Into a piece of the intestine so treated the injection mass was then introduced quite fully and the ends clamped. By massaging gently with the fingers, the injection fluid was seen to slowly fill the lymphatics.

The lymphatics of the small intestine were also very well shown by the historical method. An animal which had fasted for a day, was fed with milk. After about one hour it was killed and the intestine examined. This method showed the collectors beautifully as fine white lines which could be traced to the cisterna chyli but their network of origin in the wall of the intestine was not recognizable.

The lymphatics of the intestine arise from a very closely meshed plexus in its wall. From this network several collectors run which pass through the glands placed in their course and finally form two efferents. One of them is the efferent of the node of the descending colon, which, following the inferior mesenteric artery contributes to the formation of the lymphatic plexus around the abdominal aorta. The other is the truncus intestinalis which connects the common mesenteric node with the cisterna chyli. This latter trunk carries the lymph stream from the entire intestine, except from the lower portion of the colon.

9. *The lymphatics of the liver* (fig. 11). The deep lymphatics are easily shown when injections are made into the hepatic tissue. A number of collectors appear at the hilum and pass together with the cystic duct and the superior mesenteric vein, between the two layers of the hepato-duodenal ligament in front of the epiploic foramen and reach the retropancreatic node.

10. *The lymphatic plexus around the abdominal aorta and inferior vena cava* (fig. 16). There is a very rich, closely-woven lymphatic plexus around the great abdominal vessels extending from the bifurcation of the abdominal aorta to the cisterna chyli. This plexus is further complicated by the presence of a number of glands, scattered in the meshes. These I classified as lymphoglandulae aortae abdominalis inferioris and medialis. The following are the contributors to the formation of this plexus: efferents from the common iliac and hypogastric nodes, those from the descending colon and the collecting vessels from the kidney and testicle.

This plexus is finest in its lower course and gradually forms itself into larger trunks as it continues upwards. Near the renal vessels, behind the aorta, between the pillars of the diaphragm it becomes a large cisterna chyli, into which the truncus intestinalis empties.

8. *The lymphatics of the thorax*

1. *The cutaneous lymphatics of the thorax* (fig. 15). The ventral lymphatics cover the pectoral area. The collectors which at first are subcutaneous penetrate the panniculus carnosus and lie between it and the pectoral muscles. They join with each other as they pass toward the axilla and converge to the axillary node.

The dorsal lymphatics cover the dorsal and lateral surfaces of the thorax and also the caudal portion of the scapular region. The collectors from this area, after penetrating the panniculus carnosus converge toward the retroscapular node.

2. *The lymphatics of the diaphragm* (fig. 13). The lymphatics of the diaphragm were injected only on the ventral surface of the muscular portion. A large number of the vessels are seen to join with one another on the diaphragm just behind the xiphoid cartilage whence they continue their course as the internal mammary trunks. If the injection is made on the diaphragm a little laterally, only a few vessels appear. These traverse a few lower intercostal spaces obliquely and likewise reach the internal mammary trunk.

3. *The lymphatics of the intercostal spaces* (fig. 13). The lymphatics of the intercostal spaces were injected successfully only in the anterior (ventral) portion. The vessel from each intercostal space has a slight cephalic obliquity. It traverses the intercostal space just beneath the parietal pleura and the transversus thoracis muscle and joins the internal mammary trunk almost at right angles.

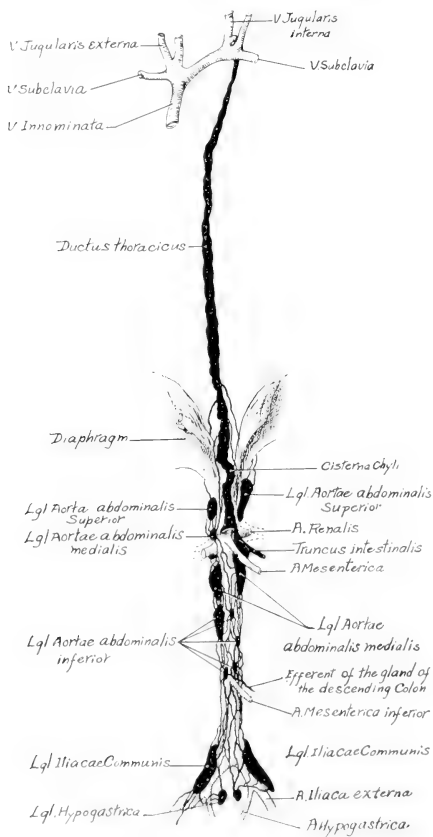
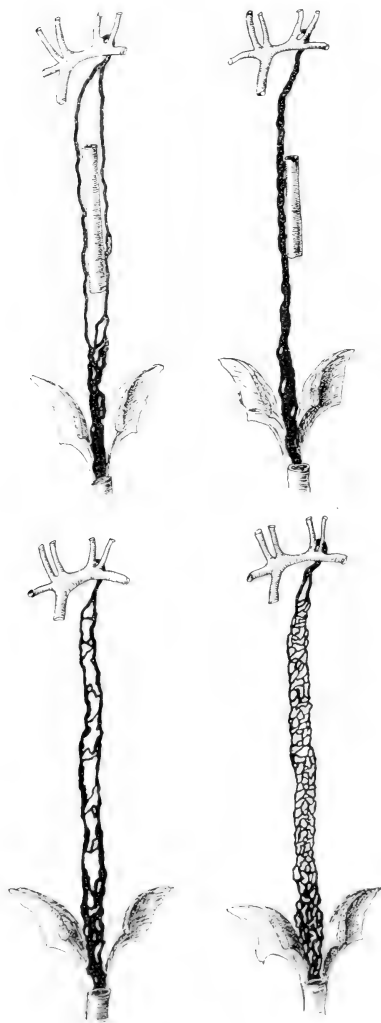


Fig. 16 The main central lymphatic nodes and thoracic duct.



Figs. 18 and 19 Types of thoracic duct

The posterior interventricular collector runs in the interventricular grooves and passes over the bifurcation of the pulmonary vein to terminate in the inter-bronchial node.

7. *Ductus thoracicus* (figs. 16, 17). The thoracic duct arises from the cisterna chyli placed between the pillars of the diaphragm, behind the aorta. In the lower thorax, it lies in the plane dorsal to the aorta and ventral to quadratus lumborum and longus coli muscles but in the upper part it changes its course and runs obliquely to the left, lying behind the arch of the aorta and the subclavian artery. From here it passes directly cephalad to reach the root of the neck, where it bends downwards to terminate at the junction of the external and internal jugular veins.

The thoracic duct presented four different types (figs. 18, 19). It is a large single trunk distinctly sacculated, which lies for the most part, on the right side of the aorta. In the upper part of the thorax it crosses the longus coli muscle to reach the left side of the median line so as to lie dorsal to the arch of the aorta.

b. Two distinct vessels may constitute the thoracic duct, one on each side of the aorta; the one lies on the left side and passes vertically to the root of the neck while the other on the right side crosses the longus coli muscle and joins with the one on the left side just behind the arch of the aorta.

c. Two vessels are placed in exactly similar manner as in the preceding but they are connected by fine anastomatic vessels lying behind the aorta.

d. The thoracic duct may be represented by a continuous network dorsal to the aorta without a main trunk. This plexus, however, terminates in a single vessel exactly as the other types of duct.

I consider myself fortunate to have undertaken this investigation under Dr. Meyer's kind direction and am indebted to him for suggestions and assistance.

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EIN PAARIGER KNOCHEN AM UNTERRAND DER SQUAMA OCCIPITALIS

ADOLF H. SCHULTZ

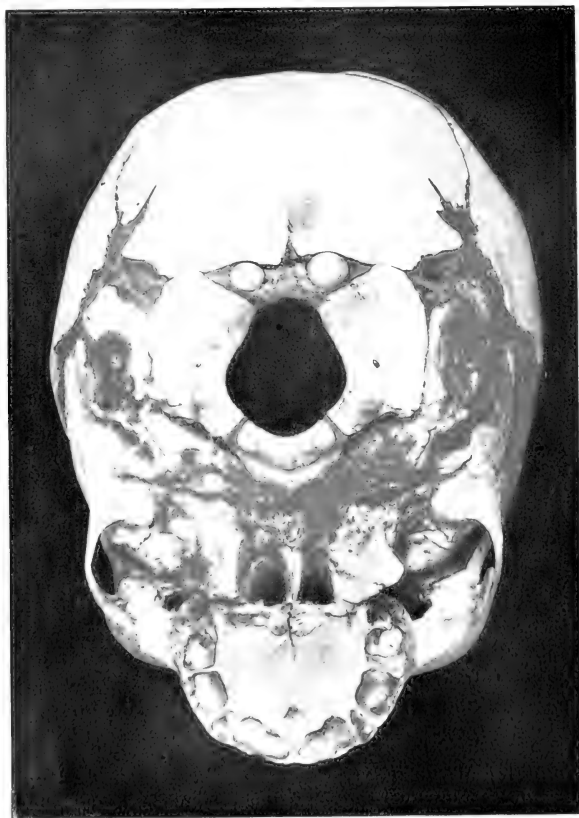
Carnegie Institution of Washington

MIT 2 FIGUREN IM TEXT

Die Entwicklung und die Anomalien der Squama occipitalis haben von jeher das Interesse der Autoren wachgerufen, wie dies aus der grossen Zahl der dieses Gebiet immer wieder von Neuem angreifenden Arbeiten hervorgeht. Selten decken sich die Resultate der verschiedenen Forscher, so dürfte denn diese Frage noch keineswegs als völlig gelöst betrachtet werden, wenn auch Aichel ('15) in neuester Zeit viel zu ihrer Klärung beigetragen hat. Von besonderem Interesse für die Entwicklung der Hinterhauptschuppe dürfte die Anomalie sein, die ich hier publiciere, da ich in der Literatur nur zwei ähnliche Fälle erwähnt fand (Weigner '12), dann aber auch, da die Anomalie die Unterschuppe betrifft und hier Abnormitäten seltener auftreten als an der Oberschuppe, ein Unterschied, der seine Begründung jedenfalls in der von Kolliker ('49) festgestellten, verschiedenen Entstehungsweise der beiden genannten Teile hat, denn während die Unterschuppe aus knorpeliger Anlage hervorgeht, bildet sich die Oberschuppe aus Bindegewebsknochen.

Der Schädel eines neugeborenen Russen aus meiner Sammlung weist am Hinterrand des Foramen magnum zwei symmetrisch gelegene kleine Knochen auf, abgesehen von dieser Anomalie zeigt der Schädel ein durchaus normales Verhalten. Wie die beigegebene Figur 1 erkennen lässt, sind die Knöchelchen in knorpeliges Bindegewebe gelagert, das sich zwischen den beiden Occipitalia lateralia und der Unterschuppe ausspannt. Dem Hinterrand der Partes laterales und dem Unterrand der Schuppe des Occipitale anliegend drängen die Knöchelchen die

ersteren vom Anschluss an die letztere ab, sodass die mediale Entfernung der beiden genannten Occipitalteile voneinander grösser ist, als dies in der Regel bei Neugeborenen der Fall zu sein pflegt. Der Rand der Unterschuppe ist an den Stellen, wo ihm die beiden überzähligen Knöchelchen anliegen, eingebuchtet. Die Knöchelchen selbst liegen 8 mm. voneinander entfernt, sind beide kreisrund und rechts 7 links 6 mm. im



FIGUR 1

Durchmesser. Während das linke Ossiculum die Gestalt einer abgeflachten Kugel zeigt, ist das rechte, grössere innen und aussen abgeplattet. Die Schuppe weist eine vom Unterrand ausgehende, kurze, mediane Spalte auf.

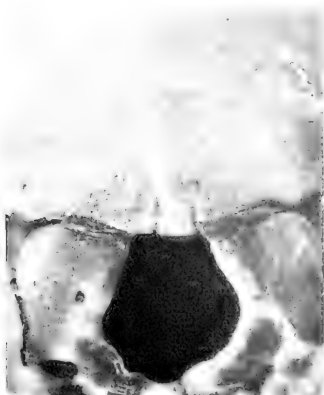
Für die Erklärung dieser allem Anschein nach äusserst seltenen Erscheinung lassen sich verschiedene Möglichkeiten anführen, aber nicht zuletzt dieser Umstand ist es, der mir, wie ich hier vorausschicken möchte, eine sichere Deutung unmöglich machte.

Unsere Knöchelchen sind dicker wie der Rand der Unterschuppe, aber ungefähr von derselben Dicke wie die Occipitalia lateralia, an deren Hinterrand sie sich anschmiegen, dies lässt es zunächst als nicht unmöglich erscheinen, dass ein genetischer Zusammenhang unserer Anomalie mit den Occipitalia lateralia besteht. Dieser Annahme wäre aber gegenüber zu halten, dass sich die letzteren stets aus je einem Stück entwickeln und das Neuauftreten eines zweiten Knochenkernes am hinteren Ende der Partes laterales des Occipitale ziemlich unwahrscheinlich ist.

Unter der Voraussetzung, dass unsere Knöchelchen der Unterschuppe des Occipitale zuzuzählen sind, lassen sich andere Erklärungen finden. Am unteren Rande der Schuppe ist nach den Untersuchungen von Lengnick ('97) vom vierten Monat des Embryonallebens an stets ein Knochen vorhanden, der später mit seiner Umgebung völlig verwächst. Es ist dieser Knochen zum ersten Mal von Kerekring (1670) beschrieben und später nach ihm als *Os Kerekringii* benannt worden. Die naheliegende Annahme, dass es sich in unserem Falle um ein paariges *Os Kerekringii* handle, scheint mir durch die Tatsache widerlegt zu sein, dass unter der grossen Zahl von als *Os Kerekringii* beschriebenen Fällen der Knochen stets unpaar auftrat, drei—oder viereckige Form aufwies und mit einer einzigen Ausnahme (Lucy '90, p. 21 " . . . une fois, nous avons rencontré cet osselet complètement libre") mit der Unterschuppe in Verbindung stand. Selten ragt das *Os Kerekringii* über den Rand der Unterschuppe hinaus,—Fälle, die Virchow (57) zu der Bezeichnung *Manubrium squamae occipitalis* veranlassten,—weit

häufiger stellt es sich als ein durch zwei seitliche Rinnen begrenzter Teil am Unterrand der Schuppe dar, wie ich dies an einer Anzahl Schädel aus dem siebten, achten und neunten Monat beobachten konnte. Ein typisches Beispiel für letzteres Verhalten ist die beigegebene Abbildung 2 des Os Kerckringii an der Occipitalschuppe eines neugeborenen Negers.

Eine Erklärung, die, wenn vielleicht auch etwas gezwungen erscheinend, so doch nicht ganz von der Hand zu weisen ist, besteht in der Deutung unserer Knöchelchen als verlagerte



FIGUR 2

Ossificationscentren der Unterschuppe. Normalerweise entwickelt sich die letztere aus zwei Knochenkernen, die schon sehr früh miteinander verschmelzen. Mall ('06) beobachtete aber an jungen Embryonen eine vierteilige Unterschuppe, wobei die vier Teile nebeneinander gelagert sind. Es wäre nun nicht unmöglich, dass auch unserem Fall eine Unterschuppe mit vier Knochenkernen zu Grunde lag, wobei durch das intensivere Wachstum des einen Paares, das andere an die Basis der Schuppe gedrängt wurde und hier die beiden kleinen Knochen entstehen liess. Wieso sich diese hier in so compacter, regelmässiger Form ausbildeten ohne mit den benachbarten Teilen zu verwachsen, vermag ich nicht zu erklären.

*Zu der letzten Deutung, die ich hier geben möchte, regte mich die Untersuchung von Weigner an, der, wie anfangs erwähnt, zwei dem unsrigen ähnliche Fälle beobachtete. Die letzteren beiden sind folgendermassen beschrieben: pg. 161. "12. Schädel eines Neugeborenen. Der dorsale Rand des For. occip. m. besitzt einen keilartigen Ausläufer, im rechten, aussen abgestumpften dorsalen Knorpel befindet sich ein selbständiges Ossificationscentrum." Pg. 163. "20. Schädel eines einjährigen Kindes. Der dorsale Rand des For. occip. m. wird inmitten von den verschmolzenen dreieckigen Knorpeln gebildet, zu beiden Seiten treffen wir wie im Falle 12 Ossificationscentren." Weigner schliesst sich der hypothetischen Annahme Kollmanns von der Existenz des Occipitalwirbels an und setzt die an Feten- und Kinderschädeln zwischen den Occipitalia lateralia und der Squama occipitalis zu findenden Knorpelplatten dem mittleren knorpeligen Teil des dorsalen Atlasbogens analog. Diese Annahme würde unseren Knöchelchen, die ja in dem genannten Knorpel eingebettet liegen, eine Zusammengehörigkeit mit der Squama absprechen, um sie dafür dem Occipitalwirbel zuzuzählen, in dessen dorsalem Bogen sie auftreten, wo sie vielleicht den Epiphysen der in der Halsregion gabligen, am Atlas und Occipitalwirbel zurückgebildeten Processus spinosi analog zu stellen wären.

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COOPERATIVE TECHNIQUE

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A realization of the very great influence that technique has upon the character of the results obtained in microscopical studies doubtless has come home to every one with extended experience in this kind of work. Time serves only to strengthen the conviction that good methods of preparation are absolutely essential to worthy results. It would be going too far to say that this conviction is universal, otherwise we should have been spared much worthless and troublesome publication, but it can safely be said that there is more general appreciation of the refinements of microscopical methods each year. Up to the present most of our processes are the heritage from the pioneers in this field who gained them through purely empirical means. Comparatively little has been added within recent years. The time would seem to have come to undertake a serious study of the problems in this field with the hope of attaining a fuller knowledge of the processes upon which we are so dependent for all progress in our investigations. It is a most hopeful sign that many biologists are devoting careful attention to this phase of their work and that more precise results are now obtainable than formerly was the case. While much of this advance has resulted from mitochondrial studies, the whole subject of microscopic anatomy has benefited from the greater precision here demonstrated.

But when the individual investigator, thus convinced of the need for advance, faces the prospect of such an extended investigation as would be necessary to developing general principles of operation, applicable to a variety of organisms and tissues, he hesitates to undertake a course which would divert so much of his limited time to methods of preparation. The usual result is that he seeks out the most satisfactory means for han-

dling his own particular materials and contents himself with the best results obtained. Considering the welfare of the whole fraternity of microscopic anatomists this is a wasteful and time consuming method, because it requires, that, in large part, each investigator must work out his own technique and when he has done so his results to a considerable degree benefit only himself.

It would seem that there is a place here for the methods of combination and cooperation which have been so effective in business operations. That this thought is in the minds of not a few biologists is apparent from the inquiries that have come to me regarding the possibility of extending the investigations that are being carried on in the Zoology Department of the University of Pennsylvania to other objects than the ones serving there as objects of study. The increasing frequency of these has led me to consider the advisability of proposing a concerted series of studies upon technical processes, applicable to as wide a range of materials as our investigators have experience with, in the hope of developing more reasonable and more generally applicable preparation methods. In connection with such a series of studies there might be built up at some central place a collection of preparations, each the work of a specialist on some group, organ, tissue or cell, from which would be loaned for study and comparison, such slides as an investigator might need. In addition the results of these technical investigations might be published either separately or collected together and reduced to a more compact form. With the method and its results before him an investigator could determine whether he had attained success in his technique. It is probable that all microscopic anatomists are interested in this phase of their work and disposed to further it, but I do not know how many are convinced that advantage would accrue from a concerted series of studies or, if convinced, are disposed, or able, to cooperate. In the hope of determining whether it is possible to inaugurate correlated studies I am writing this note with the request that any who are interested send me their suggestions upon the subject. If the plan seems feasible I shall be glad to further it in any way possible.

A SIX-LEGGED RAT

SARA B. CONROW

From The Wistar Institute of Anatomy and Biology, Philadelphia

FIVE FIGURES

During February, 1915, a male albino rat (*Mus norvegicus albinus*) with six legs and a tail in a fixed position over its back, appeared in the rat colony of The Wistar Institute. Its ancestry was not known. Its external appearance while living may be seen in figures 1 and 2, and its appearance after death in figure 3.

Two short, deformed legs hung down loosely between the two normal hind legs. They dragged on the ground and were not under the control of the animal. The base of the tail was fixed firmly in an upward curve; the rest of the tail hung loosely on one side or the other of the body and also was not under the control of the animal. There were two sets of external genitals and a false anus at the left of and ventral to the functional anus. The animal was mated but never bred. It lived for about fifteen months in good health, when, seeming to be sick, it was killed on May 26, 1916, and examined. Its body weight was 249 grams, body length 210 mm., and its tail length about 192 mm.

The anterior part of this rat was normal as far caudad as the diaphragm, beyond which there were indications of doubling. The spleen was large. On the right side in normal position was a large kidney, while low down on the left side was the rudiment of a kidney (no kidney was found in normal position on the left side). The bladder was a double structure with a perforated, intermediate septum; there was an opening through each of the two penes. On the right side were one large testis, one very small testis, and one epididymis; on the left side also were one large testis, one very small testis, and one epididymis.

Skeletal conditions in the pelvic region may be seen in figures 4 and 5.

The vertebral column remained single throughout and was normal as far as the fifth lumbar vertebra. The fifth and sixth



Figs. 1 and 2 Caudal aspect, showing normal hind legs, rudimentary hind legs, scrota and curve of tail.

lumbar vertebrae were slightly deformed and twisted. The four sacral vertebrae were more deformed; the first had the left portion of the fused pelvic girdles attached to its left transverse process, while the second had the right portion attached to its right transverse process. The six proximal, caudal vertebrae were more or less deformed and were fixed firmly in a dorsal curve which turned to the left over the animal's back. The

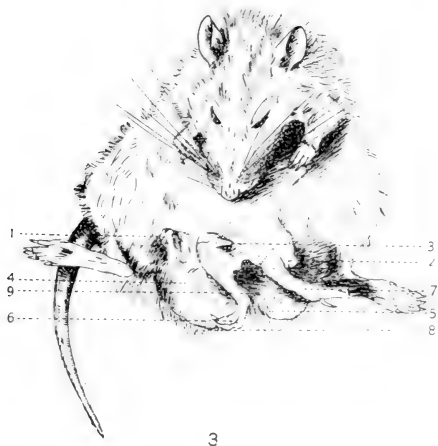


Fig. 3 Ventral aspect; 1, penis, right side; 2, penis, left side; 3, papilla; 4, scrotum, right; 5, scrotum, left; 6, right rudimentary leg; 7, left rudimentary leg; 8, anus (position under); 9, false anus.

fused pelvic girdles and bones of the deformed legs will be described later.

The structures most concerned in the duplication in this rat were the pelvic girdle, hind legs, and genital organs. The two fused pelvic girdles showed three distinct parts, namely, a right nearly normal part, a left part 2 mm. shorter than the right and proportionally smaller, and a slender, irregular bar of bone 27.4 mm. long with ends flattened dorso-ventrally (figs. 4 and 5). These three were arranged as follows: The left part held a position

further forward than the right, the crest of its ilium being 7 mm. anterior to the crest of the right ilium. The right and left parts did not meet in the median line in the symphysis pubis but their posterior ends were separated laterally a distance of more than 2 cm. Bridging this space was the slender bar of bone firmly

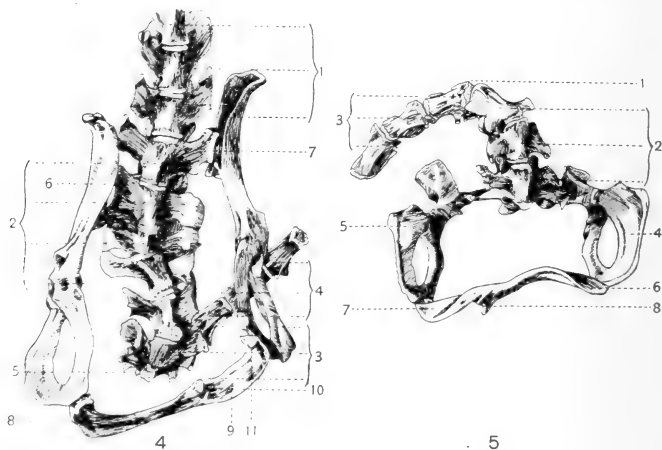


Fig. 4 Ventral aspect; 1, fourth, fifth and sixth lumbar vertebrae; 2, four sacral vertebrae; 3, first, second and third caudal vertebrae; 4, sixth, seventh and eighth caudal vertebrae; 5, curve of caudal vertebrae; 6, attachment of right part of fused pelvic girdles; 7, attachment of left part of fused pelvic girdles; 8, right deformed ilium; 9, left deformed ilium; 10, point of attachment of right rudimentary leg; 11, point of attachment of left rudimentary leg.

Fig. 5 Caudal aspect; 1, dorsal side; 2, third, fourth and fifth caudal vertebrae; 3, sixth, seventh and eighth caudal vertebrae; 4, right part of fused pelvic girdles; 5, left part of fused pelvic girdles; 6, right deformed ilium; 7, left deformed ilium; 8, point of attachment of right rudimentary leg.

attached by its flattened ends to the posterior parts of the right and left pubic bones. It seemed to be composed of two deformed fused ilia. From the ventral side of this bar hung the two short, deformed legs, one from its left end and one from a point at the left of the median line. They were attached by ligaments to slight pits in the bar at these points, and were scarcely more

than deformed feet, though each had two curved, slender bones suggesting the tibia and fibula. In addition to these bones each rudimentary leg had a deformed os calcis and the rudiment of another bone of the tarsus; in the right leg there were four metatarsal bones and four digits, all more or less deformed; in the left leg there were three metatarsal bones and three digits, less deformed than in the right, but not quite normal.

The two sets of external genitals were normally placed with reference to the two pairs of legs; namely, one set for the right normal hind leg and the right deformed leg, and the other set for the left deformed leg and the left normal hind leg. The two penes were 2.5 cm. apart and there was a fleshy papilla about midway between them. Internal conditions here have been noted.

Looking at the double portion of this animal it is seen that the right component is more fully developed than the left and that it is nearly in normal position, while the left component is wholly at the left of the median line. The two parts of the right pelvic girdle are larger than those of the left component, the right part being nearly normal and the left part being the larger of the two fused ilia and having a more pronounced acetabulum. The left leg of the right component (the right deformed leg) has more indications of tibia and fibula and more bones in the foot than has the other deformed leg. Also the functioning anus is associated with parts of the right component.

This rat apparently corresponds to the dipygus type of human monster, although its vertebral column remains single throughout. Examples of this type have been described by Gould and Pyle ('97) and by Wilder ('04). In such monsters the pelvis and the lower part of the spinal column are more or less double. Wilder ('04) describes several human monsters of this type. He gives first Wells' case of Mrs. B. Here the duplication began at the third lumbar vertebra. There were a fused double pelvis, two pairs of legs (the inner ones somewhat reduced in size), two sets of external genitals, two bladders, two ani, and two rectums. Heschel's case was of a girl seventeen years old with double parts below the second lumbar vertebra. In the case of

Blanche Dumas the pelvis appeared incompletely double and there were two extra legs, two distinct sets of genitals, and a rudimentary mamma just above the pubes. Others described by Wilder ('04) which were not strictly of the dipygus type were as follows: Jean Baptista dos Santos was wholly normal save in the pelvic region. Here there was an exact duplication of the external genitals and a median third leg, which was double and symmetrical, depended from an extra, median pelvic bone. There were four testes present. (Gould and Pyle ('97) in describing this case say that here there was possibly a double bladder communicating by an imperfect septum.) Bechlinger's case was that of a female counterpart of dos Santos. The genitals were duplicated, a third leg was attached to a continuation of the processus coccygeus of the sacrum, and there were two rudimentary mammae close together above the pubes. In the case of Louise L. there were two atrophied legs on a rudimentary pelvis attached ventrally to the normal pelvis, with two rudimentary mammae at the insertion of the legs. There was no duplication of the genital organs.

Gould and Pyle ('97), besides describing these same monsters, give also examples of differing degrees of duplication in the posterior part of both males and females, but the cases already cited correspond most nearly to conditions found in the male albino rat which has been described.

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THE FIXATION OF MAMMALIAN CHROMOSOMES

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TWO TEXT FIGURES AND THREE PLATES

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INTRODUCTION

The advance of mammalian cytology has been exceedingly slow, due partly to the difficulty of obtaining material showing the proper stages of mitosis in sufficient numbers but largely to the fact that the various methods of technique in use were not at all successful in preserving the structures of these small cells. The first difficulty is obviated by securing material from a large number of animals and examining a small portion of tissue from each animal (not more than one or two slides). Eventually a tissue may be found that is in, what might be called, a 'cycle of division,' when the mitotic figures are present in large numbers and a choice of good stages is readily made. This is true for germ as well as somatic tissue. The question of the preparation of mammalian tissue for cytological work forms the body of this paper.

During the early part of this work I had the benefit of the experience of Dr. Ezra Allen in his studies on the fixation of rat tissues (1) and Dr. McClung has assisted me all through my

experiments with suggestions and criticisms. To these men I am exceedingly grateful.

Several years ago I began a study of the chromosomes of the pig but this had to be abandoned because of the hopelessness of analyzing the chromosome complexes. Even as my figures were then, they were as good as most of the published figures of mammalian mitosis. Since unlimited material was needed for experiments on technique and since there was a fairly large supply of cats available, the early work was carried out on cat testes and the method most successful on this tissue was tried on various other mammals. For some reason I have been unable to obtain the beautiful cytological preparations that Dr. Allen gets by the use of his picro-formol-acetic-chromic mixture, although I get excellent general fixation with it. This may possibly be due to the fact that his fixing fluid was developed with special reference to the rat. With the following method, however, I get very constant results which are, I believe, comparable in every respect to those obtained by Dr. Allen. Furthermore the fixation is not only constant for one mammal but has worked with uniformly good results on seven mammals and on the somatic as well as the germ cells. With two available methods in the field the difficulties besetting mammalian cytology should be much reduced.

THE FIXATIVES EXPERIMENTED WITH

In the experiments on technique that have been carried on in the Zoological Laboratory of the University of Pennsylvania for a number of years it has been found of value to vary the temperature at which fixing fluids are used. All the fluids tried by me were used as indicated at blood heat and at about 4 or 5°C. As I had previously failed to obtain good results at room temperature I did not, except in a few instances, experiment further at this degree.

An attempt was also made to study the effect of varying the time between the removal of the tissue from the body and the placing of it in the fixing medium. In the following table the

fluids used are outlined and also the methods of applying each fixative. In many cases the experiment was repeated when there seemed any chance of obtaining better results with that particular agent.

Table of fixatives

FLUID	TEMPERATURE	TIME IN FLUID	TIME THAT ELAPSED BEFORE FIXATION
	<i>deg. C.</i>	<i>hours</i>	
Bouin.....	38 and 4	24	Immediate
B.2 + urea (1)	38 and 4	21, 23, 24 and 49	Immediate and in 15 minutes
B.3 (2).....	38	24	Immediate
B.3 + urea.....	38 and 4	24	Immediate
Carnoy.....	4	24 and 49	Immediate
Ohlmacher.....	4	24 and 48	Immediate and in 15 minutes
King's solution.....	4	4, 6, 21 and 25	Immediate
Allen's solution No. 15	38	24	Immediate
No. 16.....	38	24	Immediate
Flemming strong solution.....	Room, 38 and 4	24, 28, 30 and 49	Immediate
Flemming + urea strong solution	38 and 4	24 and 49	Immediate, 10, 15, 20, 25, 30, 45, 60 minutes and 4½ hours.

(1) and (2). B.2 and B.3 are the symbols which are used in this Laboratory to designate two modifications of the picro-formol-acetic preservative. These modifications are:

<i>Saturated</i>	<i>H.2 parts</i>	<i>B.3 parts</i>
Picric acid, aqueous solution.....	75	75
Formalin.....	10	15
Glacial acetic acid.....	10	10

To these two solutions are sometimes added 0.5 gram of urea per hundred cubic centimeters.

In controlling the temperature of the fluids I have made use of our constant temperature rooms in the case of material fixed at 38°C. When the fluids were used cold the vials containing the fixatives were packed in ice in a quart 'Thermos' vacuum jar having a very wide mouth. As this jar is provided with a handle

it is very convenient to carry about when it is necessary to go some distance for material. Flemming's solution when kept on ice registers about 4° to 5°C. Ice can be kept in this jar for several days.

The results of the experiments

The judgment that is passed on material prepared for cytological work must be based on two points:

1. *The general fixation.* If this is not good (i.e., if shrinkage or general distortion is evident) it paves the way for a very just criticism of any conclusions that may be drawn from the study of the chromosomes in such a tissue. As a rule if the general fixation is poor the chromosomes are not very likely to be decipherable, but should they appear so in shrunken and poorly fixed tissue they might well be regarded as probably abnormal.

2. *The clearness of the various stages of the chromosomes and particularly, I think, of the distinctness, differentiation and separation of the chromosomes in the metaphase plate.* In mammals, chromosomes that are more or less lumped together, even when it is apparently possible to distinguish the separate elements, do not give a true picture, in either form or number, of the actual conditions. This, I believe, I will be able to demonstrate a little later in this paper. Accepting material in such condition as workable has led several investigators of mammalian chromosomes rather far astray.

With these points in mind the material fixed by the above methods was studied. Most of the fluids used gave good preparations for general histological structure but the chromosomes were clumped and indistinguishable one from another. The first result obtained that approached the fulfillment of the required conditions was when cat testes was placed in cold (4° to 5°C.) Flemming's solution to which had been added a little urea. Previous to placing it in the fluid the tissue had been allowed to remain on a glass plate for approximately from ten to twenty minutes. Urea was added, as it had been found in the work of other members of the Laboratory to increase penetration. The

amount taken has never been accurately determined but a few crystals are added to 5 or 10 cc. of fluid bringing the concentration of the urea in the fixative probably to about 0.5 per cent. It is difficult even to surmise why this method of delayed fixation should prove successful. It might be suggested that evaporation or exosmosis might play a part in separating the chromosomes although if this were true we would expect to find considerable shrinkage of the tissue. Slight shrinkage is to be observed in tissue treated in this manner although the chromosomes appear to be in excellent condition as is evidenced by figures 3, 5, 6, 7, 8, 9 and 11. This procedure gave excellent separation of the chromosomes in the testes and ovaries from a number of cats and with the testes of a few pigs. It was successful with the only cat embryo that was fixed in this way.

It was soon found that mammalian tissue could be fixed with better results, as far as lack of shrinkage was concerned, than those obtained by the above method if the material was cut into very small pieces (not more than 2 or 3 mm. in diameter) and placed immediately into cold Flemming plus urea. It is well not only to cut small pieces but to actually tease the tissue finely in the cold Flemming solution so as to obtain rapid penetration. When pieces too large are placed in the fixing solution good results are only obtained in a narrow area around the outside of the piece. The tissue may or may not blacken when placed in the cold Flemming. The absence of the usual blackness is not necessarily indicative of poor fixation.

WASHING AND DEHYDRATION

All Flemming fixed material was washed for about twenty-four hours in running water or, when this was impractical, many changes of water were made. Dr. Allen's automatic dehydrating device (1) is a great saver of time as it does away with the necessity of handling the material so many times. In the Laboratory I use it constantly but it is impossible to make use of it in field work. I have obtained as good results by dehydrating in the usual way making the steps in the ascending series of alcohols very gradual.

CLEARING AND EMBEDDING

When the material is in 95 per cent alcohol cedar oil is added and is usually allowed to remain on the tissue at least twelve hours, one or two changes being made during this time. The cedar oil is displaced with xylol and the material is embedded in paraffin in the usual way (2).

DISCUSSION OF THE SUCCESSFUL METHODS OF FIXATION

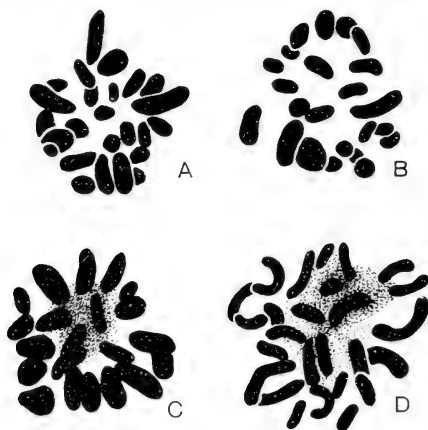
The latter method (i.e., involving the immediate placing of the tissue in the fluid) is not, as is the first, open to the possible criticism that the length of time between the removal of the tissue from the body and the placing of it in the fixing medium might allow pathological changes to enter. At present I am not of the opinion that the short wait between the removal and killing does produce anything abnormal other than a slight shrinkage. The chromosomes appear sharp and well separated and exhibit considerable differentiation. Some difference in the general morphology of the chromosomes is apparent in figures 10 and 11. Figure 10 is from the testes of a guinea-pig that was fixed immediately while figure 11 is from a testis of another individual fixed by the 'delayed' method ten minutes). The chromosomes in the 'delayed' fixation (fig. 11) are thinner and less differentiated than those of figure 10 although they are better separated. Whether this is a result of the method of preparation or not I cannot say at present. It will take considerable study and comparison to determine exactly whether this technique produces any abnormalities in the chromosomes and this study I have not completed as yet. However, since the plan of immediate fixation in cold Flemming plus urea has proved universally successful with mammalian tissue in my work, and as it is not open to the criticism pointed out above, I should suggest that it be followed in preference to the other method, using the delayed fixation only in case of the possible failure of immediate fixation.

Another point of great importance—no matter which method is followed— is that the material used must be absolutely fresh. This cannot be emphasized too strongly and is another condition

that may help to account for some of the inaccuracies in the published results of mammalian chromosome studies. Early in my work on cat testes I found that testes removed from cats that had been dead not more than a half hour and whose bodies were still warm were hopeless for a chromosome study. Recently I have confirmed this observation on the testes of ten pigs which were received after having been removed from the animals for from ten to thirty minutes. The general fixation was excellent but the chromosomes were clumped together¹ and there was no recognizable differentiation in the size and shape of the chromosomes such as is discernible in tissue which has been killed when absolutely fresh. In text-figure 1 are shown the round shapeless masses of chromatin that result from improper fixation or from the fixation of stale tissue. Such figures do not give a true picture of the actual structure of the chromosomes and it seems very likely that frequently several chromosomes may be fused thus giving an erroneous impression of the total number. I recently prepared the testes, as they were removed, from eight pigs and the fixation (immediate immersion in cold Flemming) was excellent in each case. Figure 13 was drawn from a cell in this lot of material. Text figure 2 is a photograph of a brain cell in a well fixed pig embryo. Judging from the number of chromosomes that have been reported for the pig (eighteen) (3), my own early results (about twenty-four) on this animal in comparison with my present results (over forty) it would seem exceedingly probable that a fusion had taken place between many of the chromosomes in the studies showing lower numbers. This cannot be accounted for by assuming that a difference in the breed of pig might cause the great variation as I have, among others, material from the same breed as reported on by Wodse-dalek (Poland China). Figure 3 represents a spermatogonial division in a Poland China boar. It might be suggested at this

¹ The tendency for closely associated chromosomes to fuse and appear as one when poorly fixed has been strikingly illustrated in the studies of Dr. P. W. Whiting and myself on the chromosomes of the mosquito. In these studies the actual conditions, particularly in the somatic cells, were long obscured by the tendency for closely approximated chromosomes to run together. This condition has been discussed in our papers (4 and 3).

point that staleness of tissue as well as improper technique may account for the great variation in the number of chromosomes reported for human material as it is very difficult to obtain this tissue in an absolutely fresh condition. It might also be suggested in view of the fact that all the mammals studied by me have over forty chromosomes that when a perfect fixation of human material is attained the number of chromosomes will be



Text fig. 1 All four figures represent spermatogonia of the pig in metaphase.

A and *B* illustrate the effect of poor fixation on fresh tissue. The unequal size of the chromosomes would seem to indicate that a fusion between various elements has taken place, and the massing together of these bodies in many cases make it impossible to distinguish the line of separation. Note that the chromosomes tend to be globular in form and lack differentiation.

C and *D* show the effect of the new method of fixation (immediate immersion in killing fluid) on stale tissue. *C* was taken from a testicle that had probably been removed from the animal for a longer period of time than had *D*. The large size and small number of the chromosomes of *C* again suggest that a fusion between chromosomes has taken place. The stipled area is dense and very indistinct. *D* shows a cell in a slightly better degree of preservation. The chromosomes are more numerous and better differentiated than in *C*. The form compares more favorably with the morphology of the chromosomes found in properly preserved material. In *D* there is also considerable density and lack of distinctness in the center of the chromosome plate indicated in the drawing by the stipled portion. Compare these drawings with figures 1, 2, 3, 4 and 13.

nearer that reported by Winiwarter (forty-eight) than that reported by other investigators.

The fact that a testis that remains in a dead body for a half hour or stands in a gross condition after removal from the body for a similar length of time or even less, shows the chromosomes clumped while tissue that is freshly removed, cut into small pieces and allowed to stand for ten to twenty minutes before



Text fig. 2. This is an enlarged microphotograph of the metaphase chromosomes from the brain of a pig embryo. Figure 2 is a drawing of this cell.

immersion in the killing fluid presents the mitotic figures in apparently excellent condition seems to be contradictory. For the present the fact must stand without explanation as I can offer no suggestion as to the possible reason for this behavior.

It seems probable that the explanation for the success of the cold fixation may lie in the suppression of metabolic activities when the tissue is immersed in the cold medium and the preservation of the living structures until the fluid is able to penetrate and fix them permanently.

A notable feature of the immediate fixation of mammalian tissue in cold Flemming is the perfect preservation of the extremely delicate splits between chromosomes and of the chromosome forms found in the spermatocyte divisions. These figures have seldom been demonstrated in mammalian studies. Synzesis was not seen in this material except in the center of a piece of tissue which was rather large and where the fixative had not penetrated. Even here it is not the tight ball of threads figured by so many but gives every evidence that it is the result of the extraction of fluid. In my preparations where any evidence of the synzesis of the thin chromatin threads occurs, there is only a slight contraction of the threads away from the nuclear wall appearing as though the fluid which had supported these threads had been removed. The shrinkage usually appears to be equal from all sides although occasionally a cell is found with the chromatin threads massed at one side. In well teased or small pieces of tissue these same stages appear with the threads well separated and there is no shrinkage away from the nuclear wall. That there is something different at this stage is evident but the difference appears to me to be purely a physical one.

I have been much interested in the universality in application of the two methods of fixation of mammalian chromosomes outlined above, I have obtained excellent results with them on the following tissues in the number of cases indicated under the tissue in question. The number in parenthesis under the column

	TESTES	OVARY	EMBRYO	METHOD OF FIXATION
Cat.....	13	7	1	Delayed (5, 6, 7) and immediate (17)
Rat.....	4			Delayed and immediate (14 and 15)
Guinea-pig.....	2			Delayed (11) and immediate (10)
Pig.....	13		Several	Delayed (3) and immediate (1, 2, 4 and 13)
Rabbit.....	1			Delayed (8, 9)
Bat.....		2		Immediate (12)
Mouse.....	4			Immediate (16 and 18)

headed "Method of Fixation" refer to the figures illustrating the conditions found in the material. As used above 'delayed' indicates that the tissue has been allowed to stand in open after having been removed from the body (for from ten to twenty minutes) while 'immediate' indicates that the tissue had been cut into very fine pieces or teased and plunged immediately into the cold Flemming solution upon removal from the body.

In the case of the pig most of my material has been preserved by the immediate immersion in cold Flemming's solution plus urea. I have a large number of embryos fixed in this manner and as yet have examined only a few. Each one studied has shown uniformly good fixation so far and it may reasonably be expected that the remainder will be equally good since it is fairly evident that the results are not due to a happy accident in one or two isolated cases.

THE METHOD IN BRIEF

To fix mammalian material for cytological study:

1. Obtain fresh specimens from as many different animals as possible so as to be sure of obtaining one or more in a 'cycle of division.'

2. Place small or finely teased pieces of fresh tissue immediately into cold Flemming's solution plus urea. Allow to remain twenty to twenty-four or more hours.

3. It is suggested that this second method be not used except in case of the failure of the preceding one. Allow small pieces of fresh tissue to remain in the air for from ten to twenty minutes after removal from the animal before placing them in Flemming's solution plus a little urea which is used at a temperature of about 4°C. Allow the material to remain in the fluid for twenty to twenty-four or more hours.

4. Wash in water for about twenty-four hours.

5. Dehydrate by very gradual steps.

6. Clear from 95 per cent alcohol in cedar oil followed by xylol.

7. Imbed in paraffin.

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EXPLANATION OF PLATES

I have used drawings instead of photographs to illustrate the state of preservation in the various cells since drawings show the chromosomes much more satisfactorily than do photographs in most instances. Furthermore it is difficult to find cells in animals possessing so many chromosomes in which the metaphase plate is flat enough to photograph well although it may be perfectly easy to draw the same plate. I have included one photograph, which, though not good, is probably sufficiently clear to give an idea of the separation of the chromosomes and of their large number (text-fig. 2). Figure 2 is a drawing of the same cell.

All figures were drawn using a Zeiss apochromatic, 1.5 mm. objective and the compensating ocular 12 \times which gives an original magnification of 3400 \times . These drawings were enlarged to twice the original magnification and reduced to approximately 4500 \times in reproduction.

PLATE I

EXPLANATION OF FIGURES

- 1 Prophase (late) from the amnion of a pig embryo. Immediate fixation.
- 2 Polar view of metaphase from the brain of a pig embryo. Immediate fixation.
- 3 Polar view of metaphase of spermatogonium of pig. Delayed fixation.
- 4 Polar view of metaphase from the brain of a pig embryo. Immediate fixation.
- 5 and 6 Polar views of metaphase of spermatogonia of cat. Delayed fixation.

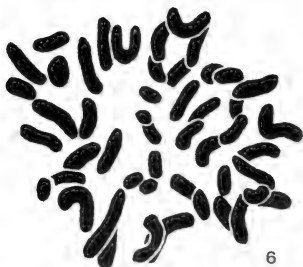
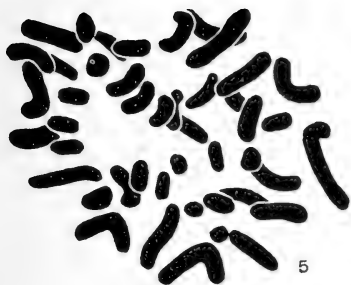
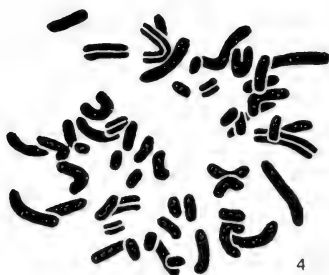
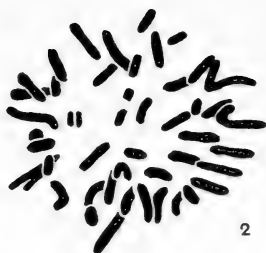
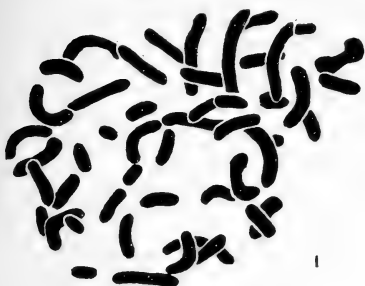


PLATE 2

EXPLANATION OF FIGURES

7 a and b Polar view of spermatogonium of a cat found in two sections. Delayed fixation.

8 Side view of the metaphase plate in the spermatogonium of a rabbit. Delayed fixation.

9 Prophase of spermatogonium of rabbit. Delayed fixation.

10 Polar view of metaphase of spermatogonium of a guinea-pig. Immediate fixation.

11 Polar view of metaphase of the spermatogonium of a guinea pig. Delayed fixation. Note the difference in the chromosomes figures 10 and 11.

12 Polar view of metaphase in the ovary of a bat. Immediate fixation.

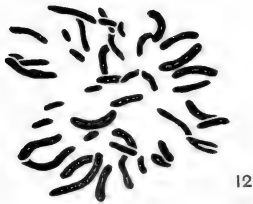
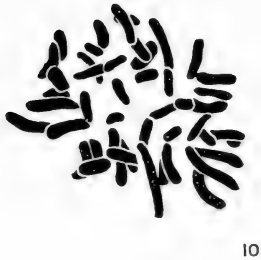
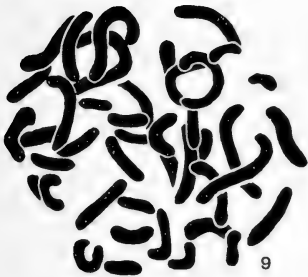


PLATE 3

EXPLANATION OF FIGURES

13 Polar view of metaphase of spermatogonium of pig. Immediate fixation.

14 Late prophase of spermatogonium of rat. Immediate fixation. This is probably a cut cell.

15 Metaphase of spermatogonium of rat. Immediate fixation. This is a cut cell.

16 Early prophase figures from a first spermatocyte of a mouse. This is only a portion of the cell. Immediate fixation.

17 First spermatocyte figures from the testes of a cat. Note the characteristic forms that have been reported for other forms but seldom for mammals. Immediate fixation. Such forms have been found in all the mammalian tissue studied.

18 A first spermatocyte from the testes of a mouse. An oblique view. Immediate fixation. This is probably a cut cell.



13



14



15



16



17



18

HERMAPHRODITISM IN *FUNDULUS HETEROCLITUS*

F. E. CHIDESTER

From the Zoological Laboratory, Rutgers College, and the Marine Biological Laboratory, Woods Hole, Mass.

THREE FIGURES

The scanty literature on hermaphroditism in fishes contains no reference to a case of simultaneous maturation of spermatozoa and ova in any species of *Fundulus*. In fact relatively few cases of hermaphroditism have been recorded in the teleosts, the only other case in *Fundulus* being in *Fundulus majalis* (Newman, '08).

The specimen to be described is interesting because it is the first known case of hermaphroditism in a species much used in experimental embryology; because it was a conspicuously well marked male in external appearance; and because the gonads which gave the secondary sex characters were entirely male and in no way attached to the egg masses. Lastly the diffuse egg masses were found in close association with the organs of digestion and in some cases occupying positions under the serous coats of the organs.

In June, 1915, while occupying a research room at Woods Hole through the courtesy of Dr. F. R. Lillie, Director of the Marine Biological Laboratory, it was desired to fertilize the eggs of a female *Fundulus heteroclitus* by the sperm of a vigorous male. A brightly colored active male measuring 7.5 cm. in length was selected from the aquarium and lightly tested for milt. The milt exuded readily and the individual was conveyed to the experimental desk and placed conveniently near in a finger bowl about one-third full of sea water, another finger bowl being laid partly over the first to prevent an escape. A few moments later, the supposed male was stripped over the selected eggs. A half dozen apparently normal transparent eggs issued from the short vas deferens. The animal was again made captive in the finger

bowl and the eggs placed therewith in the hope that sperms adherent to the body from the trial stripping might still be capable of fertilization.

A half hour later the specimen was again stripped lightly and three more eggs, a total of nine, were secured. The eggs which had previously been placed with their fraternal sperm and had been in a rather large quantity of water were examined and showed no signs of development. Four of the nine eggs (the three last secured and one of the others) were placed in a finger bowl with a very little sea water on the bottom, milt from a normal male was added to them and to a new lot of eggs from a normal female. Three hours later it was found that most of the control eggs had reached the 8 cell stage but that the eggs from the hermaphrodite had failed to develop with either lot of sperms. Later examination showed no initiation of development.

Since my stay at Woods Hole was short and it was impossible to keep the specimen under observation, it was quickly killed in Kleinenberg, then removed and opened for the penetration of the fluid. Before replacing the opened animal in the killing fluid, small portions of the testes were removed and placed under the microscope for comparison with normal testes. The sperms were apparently the same in structure and activity as normal sperms examined at the same time. Fresh testis macerated and placed with normal eggs failed, however, to initiate development.

Careful examination of the hermaphrodite was made and it was compared externally with normal males and females.

The male *Fundulus heteroclitus* reaches a length of about 5 inches and is easily distinguishable at all ages and in all seasons by the presence of a number of transversely arranged silver bars on the sides and usually by a yellowish or orange colored belly. The ground work of the body is dark green and in mature specimens at the breeding season there are numerous white and pale yellow spots of color on the sides. The dorsal fin of the male bears a dark spot at the base of the last rays; in the young male this is subdivided into two blotches. The vas deferens extends to the anal fin or even a little way along the anterior ray. The dor-

sal and anal fins bear small papillae termed by Newman ('09) contact organs, which are used in holding more firmly to the female when mating.

The young female has dark bands like the silver bands of the male and during the spawning season some older females show the dark bands against their olive ground color. The oviduct

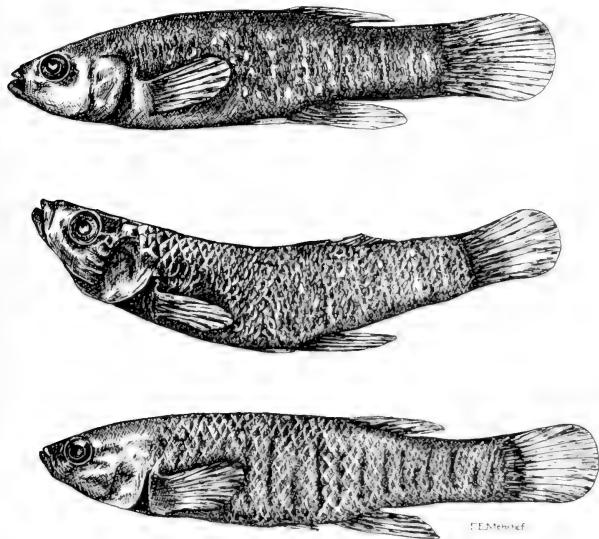


Fig. 1 A normal male, the hermaphrodite and a normal female of the species *Fundulus heteroclitus*. Drawn from a photograph by Mr. J. G. Hubbard with details from the specimens.

extends along the anterior ray of the anal fin about two-thirds its distance.

The hermaphrodite was marked like a male over the whole body except the region just behind the operculum (fig. 1).

Here the color was olivaceous like that of a female, although not noticeably dissimilar from the condition found in many males. The characteristic short vas deferens ending at the

anal fin, the contact organs, and the dark spot at the base of the last rays of the dorsal fin, all indicated male sex. Upon examination of the internal organs, however, it was easy to discover ovarian traces (fig. 2). The testes were perfectly normal in position and general appearance and likewise differed in no respect microscopically. There were a large number of diffuse egg masses attached to the stomach and intestine and studding the mesentery binding these organs. The eggs were extremely small and immature. Apparently all the mature eggs had been expressed before the internal organs were exposed. The failure of the eggs to appear at the first trial pressure is readily ex-

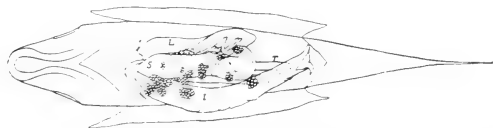


Fig. 2 The internal organs of the hermaphrodite showing *L*, the liver; *S*, the stomach; *I*, the intestine; *T*, the testes; and a large number of immature eggs. The mature eggs were extruded when the supposed male was stripped.

plainable on the ground that a little violence sufficed to break the wall of the vas deferens and allow them to pass out through the male genital aperture. It is significant that there was no indication of an ovotestis, and that all eggs visible with the binocular were closely apposed to the organs of digestion.

Sections of the testes and of the whole digestive tract with organs undisturbed from their original relations have been recently made. It was found that not only did the eggs lie closely attached to the stomach and intestines, but that they extended in small groups under the serous coats of these organs and formed nests in the muscular coats (fig. 3). It was also extremely interesting to discover that a large part of the distal end of the left side of the liver was occupied by a group of immature eggs, surrounded by liver tissue and entirely hidden from the outside.



Fig. 3 Photomicrograph of a portion of the liver and stomach of the hermaphrodite fish showing eggs. (Photograph by Mr. F. H. Dodge.

LITERATURE AND GENERAL CONSIDERATIONS

Although no case on record is like the one here described in the absolute independence of the gonadal substance, it will perhaps prove an advantage to briefly describe other cases of hermaphroditism in fishes.

Stephan ('01) describes a condition in *Sargus vulgaris*, such that there were young males with a trace of eggs in the gonads; young females with a bit of indifferent tissue representing testis; and true hermaphrodites. In regard to the production of true

males, true females and true hermaphrodites as age increased, he says:

L'age efface ces traces pour ne laisser subsister que le sexe predominant, sauf lorsqu'il y a à peu près equilibre; alors on a affaire aux hermaphrodites. . . . On remarque chez ce Poisson une grande plasticite des elements genitaux, qui fait qu'ils peuvent prendre une mauvaise direction de developpement et degenerer, ou bien remplir un rôle de secretion interne ou externe, ou quelque fonction. La plasticite et l'indépendance des divers elements sont le facteur principal qui permet d'expliquer l'apparition de l'hermaphroditisme dans des groups aussi eleves en organization.

Stephan also described certain species in which the males were precociously sexual and the females were late in maturing.

Roule ('02) found that all small individuals of certain Cyprinidae were males and all large individuals were females. He concluded that the individuals were protandric, being male when young and female when older.

Southwell ('02) described the roe of a smoked herring consisting of two parts, the anterior portion 95 mm. in length, being divided transversely into two lobes, and presenting the appearance of normal roe; while the posterior portion, lying between the two lobes of the roe in a wedge shape, was apparently normal testis 35 mm. in length. He also cites a case recorded by Smith ('70) in which the testis occupied the anterior region and the ovary the posterior region of the combined organ, and another specimen described by Mr. Yarrell in which there were two distinct lobes, one female and one male. No mention of the concomitant secondary sex characters of the specimens is included in the descriptions.

Newman ('08) has described a most interesting case of hermaphroditism in *Fundulus majalis* in which the secondary sex characters changed from female to some of those of the male during a period of one month. The external genital tube was oviducal, and the gonad was composed of about 5 per cent of testicular tissue, the rest being ovarian. The behavior changed from female to male also. About ten per cent of the eggs developed when fertilized.

Grassi ('11) described a male eel with several oocytes attached to the gonad.

Fowler (12) examined several hermaphrodite shad which had an ovotestis.

Okkelberg ('14) reported the existence in the brook lamprey, *Entosphenus wilderi* of a juvenile hermaphroditic condition normally. From a study of fifty individuals he found that 46 per cent were true females, 10 per cent were true males and 44 per cent were true hermaphrodites. Fifteen adult males were examined and of these seven contained undeveloped ova attached to the testes. The conclusion is that all the hermaphrodites develop into males.

The specimen of hermaphroditism here described is quite different from any of those hitherto recorded. It has diffuse egg masses extending from the stomach to the posterior portion of the large intestine, yet no single egg mass occurs nearer than the opposite side of the intestine to the testes. The suppression of further ovarian growth in other cases recorded is quite easy as the testis and ovarian substances are in close apposition. The wide distribution of the egg masses in this case is probably due to some environmental distribution of the anlage.

Persistence of the eggs even to apparent maturity, is rather difficult to explain if we attribute to the fish the same regulatory power possessed by the gonad of the mammal.

It is very evident on the other hand that an appreciably large per cent of ovarian tissue scattered through the body of a fish has no power to influence the formation of the secondary sex characters. In other cases recorded, notably the one described by Newman ('08) the presence of gonadal substance of the opposite sex in very small quantity, but in connection with the dominant gonad, was sufficient to prevent the full assumption of secondary sex characters. Any explanation of the condition we have described must recognize the evidence for an internal secretion produced by a regularly formed organ but absent in diffuse ovarian masses.

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REFERENCE MODEL OF THE THORACIC VISCERA

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THREE FIGURES

Recently a model of the abdominal viscera (Bellevue Model No. I) made by casting directly from a formalin hardened subject was described in the Record (vol. 10, p. 591). Adopting the same procedure, casts were made from the thorax and part of the neck of another subject. In the present case the subject used was a well-developed female who died of double lobar pneumonia, involving the lower lobes, at the age of thirty-four. The lungs showed no evidence of antecedent disease. There were a few soft pleuritic adhesions from the pneumonia of which the subject died. These were very slight and scarcely modified the natural surface of the pleurae.

The model consists of the lungs and of a stand comprising the cupolae of the diaphragm, the dorsal wall and diaphragmatic part of the pericardium, the lower part of the oesophagus, the larynx, trachea, bronchi, thymus and thyroid.

As in the abdominal model, the casts are made of a light plaster composition. The model complete is now sold by the maker uncolored but it is expected that casting in colors will soon be possible. In this case the colors, of course, will be permanent and will not be lost through wear, as in a painted model.

The model is represented in figures 1, 2, and 3, reduced about one-half. Figure 1 shows the medial surfaces of both lungs; figure 2, the entire model, posterior view; figure 3, the stand, anterior view. The following notes will explain the topography and anomalies of the dissection.

Lungs. The lungs show all the usual impressions on the mediastinal surface with the exception of that for the oesophagus near the base of the left lung. In the hilum, the cut sur-

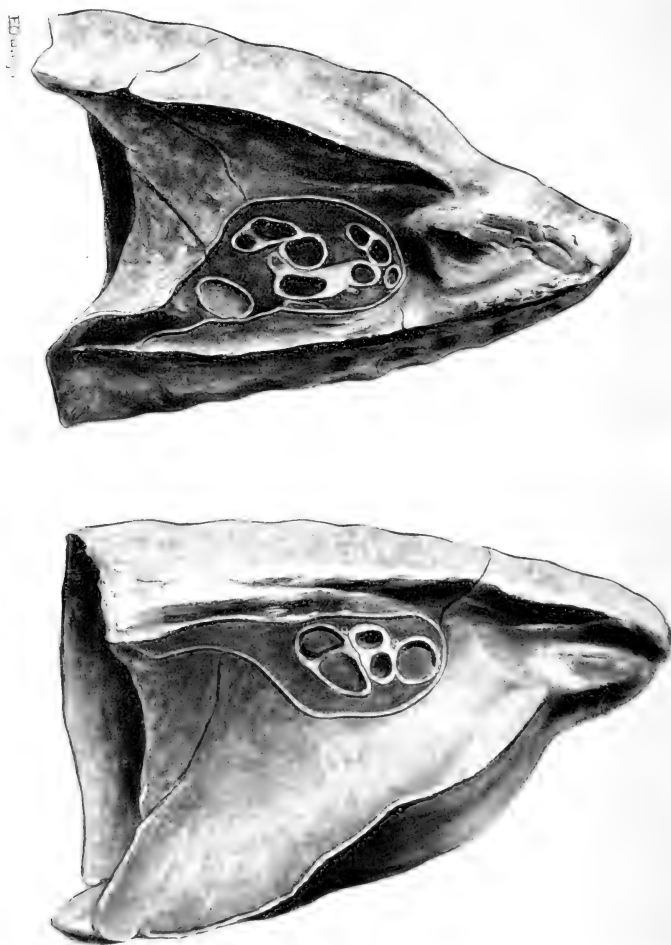


Fig. 1

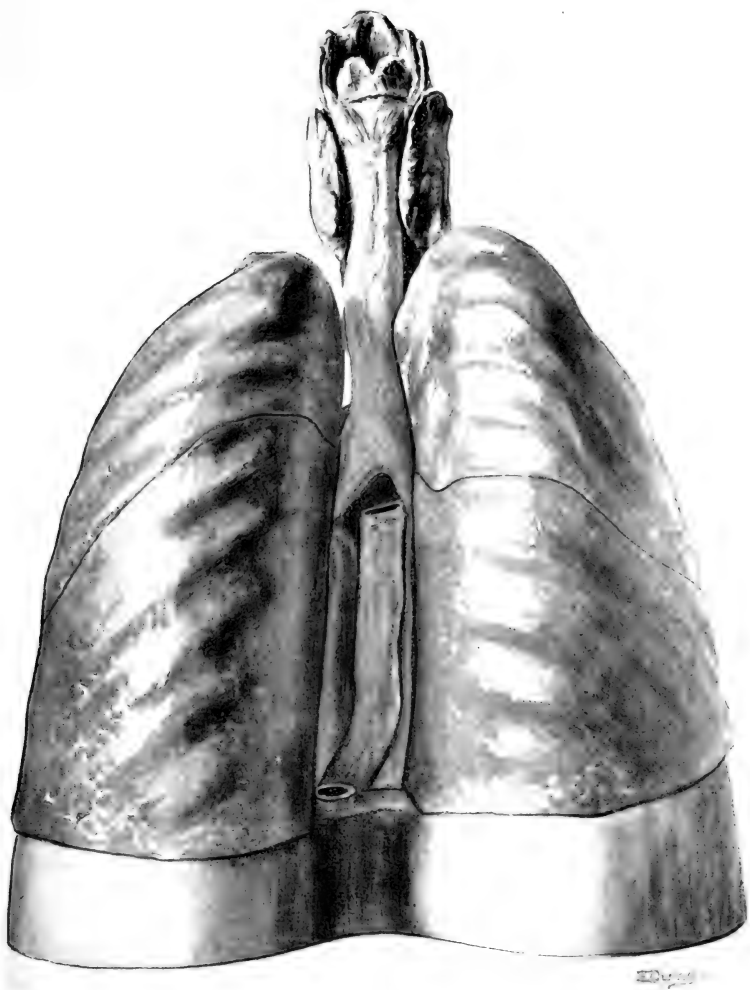


Fig. 2



Fig. 3

faces of the pulmonary arteries and veins and the bronchi are clearly marked. In the right lung the bronchus is dividing into eparterial and hyparterial parts, the latter giving off a branch which passes downward and forward. A right bronchial artery is shown passing to the posterior surface of the bronchus. The main stem of the pulmonary artery lies in front of the hyparterial bronchus while above it is the branch accompanying the eparterial bronchus. This branch is already subdividing into several smaller branches. The upper right and lower right pulmonary veins are in the usual positions, the former already dividing. In the hilum of the left lung the pulmonary artery, the bronchus and the pulmonary veins lie in the order named from above downward. The veins are uniting to form a common left pulmonary vein before entering the pericardium as shown in the stand. The left bronchial arteries, cut short, appear one above and one below the posterior part of the bronchus.

The ridges and furrows corresponding to the bony and muscular parietes are possibly accentuated by inflammatory swelling. These are unusually distinct. Some of the smaller vessels and nerves have left more or less distinct furrows upon the lung surface; thus there are impressions corresponding to the sympathetic cord and ganglia, the splanchnic and phrenic nerves and the internal mammary artery.

With regard to the furrows upon the costal surfaces of the lungs: The axes of these in the ventral region correspond to the main longitudinal axes of the ribs. As the furrows are traced dorsally, they may correspond either to the margin of a rib or to the internal intercostal muscle which frequently bulges beyond the level of the contiguous ribs. In order to map out upon the lung the areas occupied by the costal arches, it has been necessary to place the thoracic wall accurately in position upon the cast and then to mark accordingly. An old fracture of the right third, fourth and fifth ribs, in which union has occurred without accurate apposition of the bones, has left its impression upon the corresponding lung.

The stand. The dorsal portion of the pericardium remains in situ upon the diaphragm which forms the base of the model. In removing the heart, the vessels have been cut in such a manner as to indicate the lines of reflection of the serous pericardium around them. The left pulmonary vein is a single vessel at its point of entrance into the pericardium. The greater part of the aortic arch and descending thoracic aorta have been removed. The space occupied by these is clearly indicated when the left lung is placed on the stand. In the interior of the pericardium, the oesophagus and descending aorta have produced a broad vertical ridge between the lower right and common left pulmonary veins. The left portion of this ridge which corresponds to the aorta, is the more prominent and extends further upward.

The thymus gland was long enough to overhang the cephalic end of the pericardium. In order to reduce the complexity in this region, the right and left lobes of the gland were folded upward and secured in position before casting.

The pharyngeal mucosa has been left intact over the dorsal aspect of the arytaenoids and over the ventral and dorsal aspects of the upper part of the epiglottis. The dorsal aspect of the cricoid is partially covered by the posterior crico-arytaenoid muscles.

The model was prepared under the direction of Prof. H. D. Senior and the writer, Department of Anatomy, University and Bellevue Hospital Medical College. It is called "Bellevue Model No. II, A." All inquiries as to price, etc., should be addressed to Professor Senior. Orders may be addressed to G. Von Bouchaute, care of the above department.

NOTES ON THE PREPARATION OF BONES FROM MADDER-FED ANIMALS

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Past work upon bones of growing animals which have been subjected to a short course of madder-feeding has been done principally with dissected dried specimens and sections prepared by grinding and polishing thin slips. Decalcification, of course, decolorizes the dye, so that satisfactory sections cannot be made in the ordinary way. During some recent investigations with these vitally stained bones recourse was had to several methods which were found to be helpful in bringing to light the morphological details.

Of these methods perhaps the most useful was the familiar one of Spalteholz ('14), recently mentioned in this journal by Shipley and Macklin ('16) in connection with the study of bones vitally stained with trypan blue, and by Noback ('16) for the clearing of preparations of stained cartilage. Its advantages for the study of madder-bones are the sharpness with which the most rapidly growing areas of the bone are marked out, the clearness of the bony structure, and the ready means afforded of looking into the depths of the osseous tissue as well as upon the surface.

The technique is very simple: the specimens, fixed in 10 per cent neutral formalin, washed and cleaned, are dehydrated thoroughly in alcohol and treated with benzene followed by oil of wintergreen. Small bones, such as those of the rat, are particularly well suited to this method of treatment. In these, with the aid of the binocular microscope, the finer structure of the bone can be seen. The larger bones may be cut or sawn into thin sections before being cleared. In these the details are very distinct.

In bones from growing animals which have been fed with madder for a short time the areas of most rapid growth, as the ventral ends of the ribs, or the epiphysal lines of the long bones, are seen to be most densely stained. The region close to calcifying cartilage, where new spicules of bone are being formed, shows a fine velvet-like surface, while farther back on the shaft the trabeculae become coarser and the structure more open and plexiform. A cylinder of red, denoting the new bone formed in the process of periosteal ossification, characterizes the shaft of the bone. The stained centers of ossification are clearly outlined and their structure may be studied.

Membrane bones are strikingly shown by this method, the suture lines presenting a serrated edge of more densely stained bone, the tooth-like processes being quite red. The dye-deposits follow largely the lines of the blood vessels. The central cores of the teeth are well stained, but the enamel remains uncolored.

Beautiful preparations may be made of the bones of animals subjected to prolonged feeding with madder. A number of rats were fed in this way continuously from birth for five months (the dye being given at first in the mother's milk, with the result that the bones were colored throughout, as was to be expected, since all the calcium-salts laid down during the extra-uterine lifetime of the animal are impregnated with the dyestuff.

Sharp contrasts are presented where the normal feeding had been resumed for a few days, following a short term of madder-feeding, in a growing animal. Here the red bone stands out very clearly against the white bone which is deposited last, and which now caps the growing ends and lines the edges of the bones. Under higher magnification the relations of the colorless to colored bone in the trabeculae may be studied. Striking pictures of the way in which the bone grows and is absorbed are thus afforded.

Madder-stained pathological calcareous deposits may also be cleared by this method, and provide instructive specimens. The same is true for the callus formed in the repair of bone injury.

Permanent mounts in Canada balsam or damar may be made.

The Schultze method may also be used with good results in preparing these bones, and is especially to be recommended for entire specimens, where it is desired to retain the original outline. Embryos whose bones have been vitally stained by feeding madder to the mother have been successfully cleared in this way. The 1 per cent solution of KOH was employed, as recommended by Mall ('06). For the finer details of bone structure the Schultze method is not so good as that of Spalteholz.

In preparing dried specimens of madder-bones the method of maceration was employed, and was found to be very valuable in facilitating the cleaning of the skeletons. For the details of this method I am indebted to Miss Sara B. Conrow of The Wistar Institute, who uses it in the study of the unstained rat skeleton. The animal, having been skinned and eviscerated, is put into a solution of an alkali at a temperature of 95°C. to 97°C. and maintained at this temperature until the soft parts can be easily removed. A 0.2 per cent solution of KOH gave good results. Miss Conrow obtains splendid results with a hot 1 per cent or 2 per cent solution of ordinary 'Gold Dust' washing powder (a 2 per cent solution being used for adult rats). The young rat, after skinning and evisceration, is placed whole in the hot 1 per cent solution in the hot oven; with older rats, however, the larger masses of muscle are removed and a number of the bones disarticulated and placed in separate containers in the oven.

The length of time in the oven depends on the age of the rat and on how much muscle has been removed from the bones, though much more on the former. The young rat should be watched at intervals and the feet, when they soften, removed to shallow containers of tap water, to prevent their dropping off and the bones becoming mixed. The adult rat should be watched at the end of one hour in the oven and the condition of the flesh tested with a forceps. The soft parts should be so much softened that the bones will clean easily in tap water by the use of a small forceps, a tooth brush, a small bone scraper and perhaps a scissors.

If it is desired to maintain the numerous elements of the skeleton in their proper relationships one to another, the bones of the right and left sides of the body may be placed in separate containers in the oven and care taken in the cleaning to keep the bones in order.

The various bones, after being cleaned, may be laid out in their proper order on stiff cardboard and may afterward be mounted on glass plates if desired.

Bones prepared in this way are of a brilliant red color, the alkali serving to intensify the madder-dye.

The method of digestion, at 40°C. in a mixture of 1 per cent pepsin and 0.2 per cent HCl in distilled water, to which a little thymol was added to allay putrefaction, was tried, but was slower and the results were not so good as in the case of maceration in an alkaline solution, the effect of the acid being to decolorize the dye.

It is best to store the preparations in a dark place.

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A CULTURE MEDIUM FOR EUGLENA WITH NOTES ON THE BEHAVIOR OF EUGLENA

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I. INTRODUCTION

Quince seed jelly has been in common use as an agent for retarding the movements of Protozoans but as far as the writer can determine it has not been suggested as a culture medium.

While working with quince seed jelly to retard the action of the flagellum of *Euglena* it was discovered that *Euglena* would live for several days in the medium. *Paramoecium* also existed for several days in depression slides surrounded by the medium. It was conceived that Protozoans might be kept alive for a considerable length of time and an attempt to test this possibility suggested the value of further experimental work.

It was found that not only would *Euglena* live in the medium but it seemed to obtain nourishment from the medium and increased in great numbers. Cultures have been kept for fourteen months. Two hundred successful transplants have been made from a single culture and *Euglena* has been available for class work at all times throughout the year. The following experiments have been carried out to determine the best conditions for raising *Euglena*.

II. EXPERIMENTS TO DETERMINE THE OPTIMUM CONDITIONS FOR THE DEVELOPMENT OF EUGLENA

1. *Acidity and alkalinity*

Experiment a. Quince seed jelly was used which had been exposed to the air for several weeks and had acquired a strong acidity evidently through fermentation. Twenty-five tubes of the medium were prepared and inoculated with cultures of *Euglena* which had been grown on a neutral medium.

Six of the cultures contained live *Euglena* at the ends of three weeks. Two cultures contained *Euglena* at the end of six weeks and all were dead at the end of twelve weeks. Moulds were abundant in the cultures after five days. In three of the cultures a mould developed which contained a brilliant orange pigment.

Experiment b. Acid medium from the same stock as that used in the preceding experiment was neutralized with sodium carbonate.

Twenty tubes of the medium were inoculated with *Euglena* from the same culture as in the preceding experiment.

At the end of three weeks all the cultures were living and only three were dead at the end of six weeks. Sixteen of the twenty cultures were alive at the end of twelve and all had shown a great increase in the number of *Euglena*.

Experiment c. Twenty test tubes were prepared each containing 50 cc. of neutral medium. Five were kept as controls while amounts varying from one to seven drops of $\frac{N}{10}$ sodium carbonate were placed in the others. They were inoculated with *Euglena* from a neutral culture.

All cultures were alive at the end of two weeks but no marked difference in their progress could be distinguished. At the end of four weeks there was a slight difference in favor of the more alkaline cultures. After a period of seven weeks all the cultures were flourishing. There was no perceptible difference in cultures differing only slightly in their alkaline content but the most alkaline had progressed somewhat more than the neutral and the slightly alkaline ones.¹

Experiment d. The acid medium used in experiments a and b was used here. Fifty lots of media of 50 cc. each were prepared and arranged in ten equal groups. (The medium at this time had a total acidity which required 8 drops of $\frac{N}{10}$ sodium carbonate to neutralize 50 cc). Five cultures were kept on the acid medium as controls. Amounts of sodium carbonate varying from 3 to 17 drops were placed in the other lots. Group number one (the controls) were thus rather acid while group number ten was equally alkaline. Group number five was about neutral. *Euglena* from a 'Wild' culture were used to inoculate the tubes.

After six weeks active *Euglena* were found in the slightly acid, the neutral and all the alkaline cultures. In the alkaline cultures there had been a substantial increase in the numbers of individuals. No *Euglena* were found in the strongly acid cultures but a few encysted individuals were found in the cultures having a moderate acidity.

Experiment e. In cultures kept for a year in a thick jelly it was noticed that the medium was reduced to a thin liquid having the consistency of water wherever the animals had remained in one place for some time. This liquid was tested from time to time and gave a strong alkaline indication while the medium at the center of the tubes was still neutral. The liquid part of the cultures increased in its alkalinity with the age of the cultures. It is not necessary to postulate a specific substance in the decomposition products of *Euglena* to account for this liquefaction. A thick jelly may be reduced to a smooth liquid by ammonium hydrate or sodium carbonate and conversely a thin medium may be set to a jelly by a strong acid.²

¹ In tests for alkalinity and acidity $\frac{N}{10}$ and $\frac{N}{20}$ solutions of sodium carbonate and of hydrochloric acid were used with alizarin as an indicator.

2. *Light*

Cultures kept in a moderate light were successful when other factors were equal. Cultures kept in the sunlight died in a short time. *Euglena* will not live in the dark.

3. *Density*

Experiment a. Two cultures were started in the fall of 1915 in very thick jelly. No effort was made beyond a preliminary boiling to keep the tube sterile. About five hundred individuals were placed in each culture. At the end of three months each culture showed a conspicuous green color at the top. After six months the green color was present at the tops, sides and bottoms. The medium at the top, sides and bottom was thin while that at the center of the tubes was unchanged. At the end of nine months about a third of the entire volume of the medium had been liquefied and this zone was occupied by enormous numbers of *Euglena*. Over two hundred transplants were made from one of the cultures at this time. At the end of the thirteen months both cultures had shown a still greater increase in numbers. The liquid zone had increased and as previously stated gave a strong alkaline reaction, whereas the original medium had been neutral.

Experiment b. Forty cultures were started under the same conditions as in experiment a.

After five weeks twenty-eight of the cultures had started through the course just described in experiment a. Twelve were dead and a thick growth of mould had taken possession of them. Most of the dead cultures displayed a slight acid reaction.

Experiment c. Media was prepared by evaporating the jelly to dryness and making up solutions to the desired consistency with distilled water. The following dilutions were made up: 0.75 per cent, 0.5 per cent, 0.4 per cent, 0.3 per cent, 0.25 per cent, 0.2 per cent, 0.1 per cent, 0.05 per cent, 0.025 per cent. Forty cultures were started with *Euglena* from a 'Wild' culture, five cultures upon each of the above named dilutions. Test tubes having a bore of 8.25 mm. were used.

At the end of a few weeks a few inactive *Euglena* were found in the cultures presenting a 1 and a 0.75 per cent dilution. A growth of mould had started at the tops of some of the cultures. In all the other cultures there was a downward migration, all the *Euglena* being arranged in a horizontal plane not more than 0.5 mm. in width. The extent of this downward migration varied directly in proportion to the density of the medium.

Dilution per cent	Vertical distance of migration mm.
0.5	2.8
0.4	3.25
0.3	3.6
0.2	4.25
0.1	8.0
0.05	11.0
0.025	17.0

At the end of two months only a few slowly moving and encysted individuals were found in the cultures representing a 1 per cent, a 0.75 per cent and a 0.5 per cent solution. In the others the migration had reached the bottoms of the tubes and there had been a great increase in the actual number of *Euglena*.

Heat and cold

Sixty cultures were prepared, twenty being in an incubator at 37°C., another twenty kept at room temperature and the rest in a temperature of about 7°C. Those kept at room temperature produced flourishing cultures. The increased heat of the incubator tended to promote bacterial growth and to dry up the medium. Those kept at 7°C. were slow to develop but produced good cultures when returned to room temperature. The lower temperature was favorable for the growth of mould.

5. Shape of receptacle

Cultures made the most rapid progress in test tubes, vials and jars where the *Euglena* had a considerable vertical range. Cultures were also tried in flat bacteria dishes where there was a large surface exposed. These cultures were generally failures.

6. Sterile cultures

About 70 per cent of all cultures attempted with no precautions as to their being sterile were successful. Sterile media can be easily prepared, however, by boiling and cultures planted in sterile media give more uniform results. It is very difficult to keep cultures sterile as the introduction of *Euglena* can hardly be accomplished without giving access also to some bacteria.

7. Bacteria and moulds

The presence of a small quantity of bacteria does not seem to materially affect the progress of a culture except when the cultures are kept at a temperature of more than 21°C. The presence of moulds not only retards the progress of the culture, but the mycelium makes it difficult to follow the progress of *Euglena*.

Cultures containing a dense medium were found to contain a thicker growth of mould than those grown on a thinner medium. Experiments proved that a medium representing a 0.25 per cent solution of dried jelly in distilled water will not support a growth of mould. Neutral and slightly acid cultures usually contained growths of mould but the alkaline cultures, whether weak or strongly alkaline were free from it. Old cultures of *Euglena*, grown on a thick medium, will automatically clear themselves of mould by becoming alkaline.

8. *Effects of transplanting*

No weakening effects have been observed in transplanting cultures and frequently a slowly developing culture in which there is a tendency for the *Euglena* to encyst will become rejuvenated by being transplanted.

9. *Media prepared from ground seed*

A tendency to group about pieces of seed was exhibited by *Euglena* in some early cultures. An effort was made to test this tendency to see whether it might be of practical use. Seed was ground to a fine meal and a medium prepared by adding water in the proportion of 20 grams of ground seed to 2.5 liters of water. This medium produced the most favorable results for the rapid development of a culture. Fifty cultures of 50 cc, each were started, using this medium. About a thousand *Euglena* were placed in each culture. At the end of six weeks the numbers had increased till the entire volume of the cultures had assumed a bright green color and it was estimated that each culture contained 15,000 individuals per cubic centimeter.²

III. NOTES ON THE BEHAVIOR OF EUGLENA

The usual positive phototropism in exhibited *Euglena* shows a marked difference in its behavior in thick and thin media. Whatever the density of the medium there is a persistent effort to migrate downward. In thin jelly locomotion by means of the flagellum is possible and the downward migration is accomplished by this means. During this migration all the animals are to be found in a narrow horizontal zone as described under 'density', experiment three. In a very dense medium locomotion can take place only by means of 'euglenoid' motion and this is relatively ineffectual for progress unless the animal is in contact with a solid surface. The behavior described under 'density', experiment one can be explained according to this observation. Here, only the tops, sides and bottoms of the tubes are occupied or only those regions which can be penetrated by euglenoid locomotion.

² In estimating numbers a known quantity of media with the *Euglena* was diluted to a known volume. The tube was thoroughly shaken to insure an even distribution of the animals. A small measured amount of the medium was then placed under a binocular and the *Euglena* counted.

The density of the medium thus plays an important rôle in the behavior of *Euglena*, both in determining the mode of locomotion (whether euglenoid or flagellate) and second, in limiting the distance of migration within a given time.

IV. CULTURES OF OTHER ANIMALS IN QUINCE SEED JELLY

A minute green flagellate appeared in some of the *Euglena* cultures which had been inoculated from 'Wild' cultures. Transplants have been made and pure cultures obtained. So far these cultures have lived for four months and a half and are reproducing rapidly.

A large spiral shaped Bacterium was found to reproduce in great numbers in a slightly acid medium that had been kept at room temperature.

Paramoecium and a large Stylonychian have been kept for three months in mixed cultures. They feed upon *Euglena* and the bacteria.

Two small species of Rhizopods appeared in some mixed cultures and lived for about four weeks. An attempt was made to raise *Amoeba Proteus* in the medium but without success.

Nematode worms have been kept alive for a period of six weeks in the medium.

Mosquito larvae lived for three weeks in a rather dormant condition.

The medium seems to serve as food for the chlorophyll-bearing Protozoans and for some bacteria. The other animals live only as long as food is furnished in the form of *Euglena* and bacteria.

V. FLAX SEED JELLY AS A SUBSTITUTE FOR QUINCE SEED JELLY

A jelly may be obtained from flax seed by boiling the whole seed in water for a short time. At the date of this writing cultures of *Euglena* have been kept for five weeks in this medium and seem to be thriving quite as well as those in the quince seed jelly.

VI. PREPARATION

1. Quince seed jelly may be prepared quickly for laboratory use in the following manner:

Boil 20 grams of dry quince seed in 1.5 liters of distilled water for about half an hour, stirring occasionally. A gelatinous exudate will be obtained from the seeds. Pass the water containing the exudate through a wire sieve to remove particles of seed. (An Eimer and Amend sieve number 80 is fine enough to clarify the jelly and still permit it to pass through freely). Make up the volume to two and a half liters with distilled water. The medium will keep for several months if sterilized.

2. It is possible to obtain for experimental purposes a medium having a constant as regards density and chemical content by evaporating the jelly to dryness and making up standard dilutions with distilled water.

3. *Euglena* have been found to develop most rapidly on the following medium: ground quince seed, 20 grams; distilled water, 3 liters.

The above methods are practical for the laboratory because they require no special technique for their preparation and the cultures need but little attention when once started.

VII. SUMMARY

1. *Euglena* have been kept for over a year on a medium composed of quince seed jelly and water.

2. For cultures expected to last for a long time a thick jelly which has been rendered slightly alkaline should be used. Cultures planted on such a medium develop slowly.

3. Cultures develop more rapidly on a thinner medium.

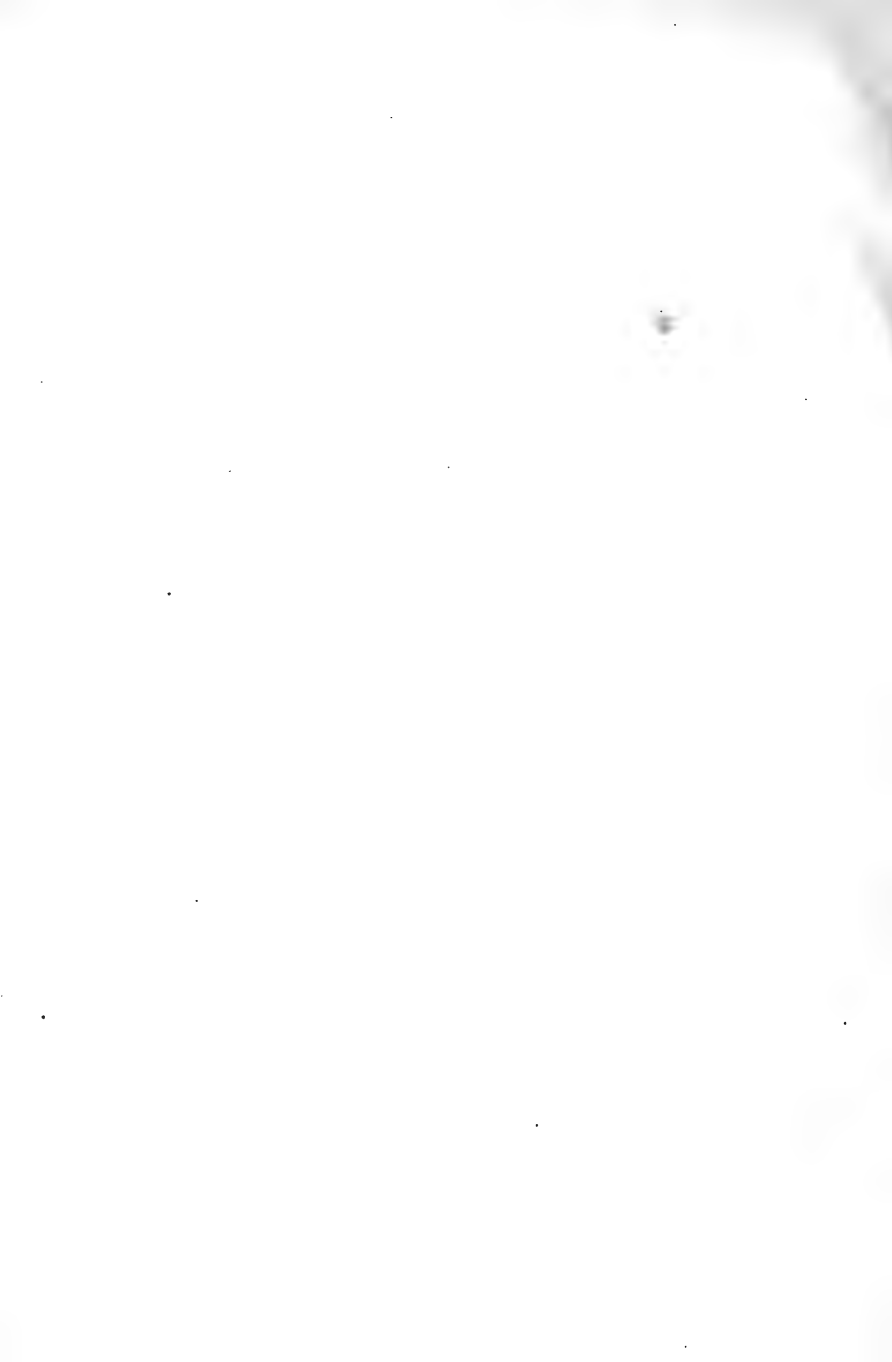
4. Cultures develop most rapidly on a medium composed of ground quince seed and water. This medium has the disadvantage of being opaque.

5. Cultures should be kept at room temperature, in an moderate light and in glass containers having a considerable vertical range.

6. Other Protozoans have been kept for several months in the medium.

7. The behavior of *Euglena* is modified by the medium.

8. Transplants can be made successfully.



THE ACTION OF VITAL DYES IN TELEOSTS

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The demonstration, by Bouffard ('06) and by Goldmann ('09), showing that animal tissues may be stained *intra vitam* by the injection of solutions of certain benzidine dyes, quickly led to the recognition of the fact that the body possesses scattered groups of closely allied mononuclear cells, capable of absorbing and storing colloidal substances of all degrees of dispersion. These related cells, possessing the ability to ingest and house particulate matter, have been variously designated as macrophages, clasmatocytes, pyrrhol-cells, endothelial leucocytes and histiocytes.

The benzidine dye-stuffs, forming, as they do, ultra-microscopic solutions, many of which are practically nonirritant to living tissue, afford ideal conditions for the study of this class of cells and have served generally for their recognition. With a knowledge of the action of these dyes a reinvestigation of many normal and pathological processes has become necessary.

A comparative study of the distribution and fate of vital-dyes in the different groups of vertebrates, though important, has never been undertaken. Outside of mammalia, the behavior of vital-dyes has been little observed; this is due to the fact that mainly clinicians have been interested in the problem of vital-staining.

It is the object of the present paper to present some observations on the absorption, distribution and excretion of vital-dyes in the bony-fish, and in doing so, to furnish additional data whereby a comparative study of the cells affected by these substances may be facilitated.

There are a number of publications by Metchnikoff and his coworkers dealing with certain aspects of phagocytic activity in

fish. Metchnikoff ('05) was aware of the fact that red blood-corpuscles of the guinea-pig, when injected into the peritoneal cavity of gold-fish (*Cyprinus auratus*), were quickly seized upon and phagocytized by mononuclear cells inhabiting the serous cavity. His pupil, Mesnil ('95), while studying the reaction of teleosts (*Perca fluviatilis*, *Gobii fluviatilis*, *Carassius auratus*) towards injected virulent anthrax bacilli, demonstrated that the pathogenic organisms were ingested and rendered innocuous by numerous mononuclear cells. These elements Metchnikoff refers to as haemo-macrophages, averring that they are related to other groups of mononuclear phagocytes resident in the spleen, bone-marrow and lymph-nodes; and he describes them "as leucocytes with abundant protoplasm which stain readily by basic aniline dyes, whose nucleus, however, is sometimes divided into lobes." The author finds no other literature concerning the mononuclear phagocytes of fish.

The fish used for the following investigations were a species of carp, common to the fresh waters of the United States and originally introduced from Europe; they belong to the family Cyprinidae and genus *Carassius*, and, as nearly as could be determined, are closely allied or perhaps identical with *C. auratus*. The animals are an olivaceous color, their length averaging 15 centimeters, their weight 75 grams. They are extremely hardy and may be kept for months in the laboratory in aquaria supplied with running tap-water.

One cubic centimeter of a 1:100 solution of trypan-blue, was injected into the peritoneal cavity of each fish with as nearly sterile technique as possible. The whole procedure took less than a minute and the animals seemed absolutely undisturbed by it.

The first noticeable effect appears a few hours after the injection of the coloring matter, and consists in a gradually developing blue coloration of the integument, an indication of a rapid absorption of the dye from the peritoneal cavity.

The mode of absorption into the circulation from the peritoneal space appears to be by diffusion of the solution through the walls of the blood-vessels. Although there seems to be a slow

diffusion of the injected fluid through all the peritoneal surfaces, there is most probably a selective absorption by vascular tissue; for, if the peritoneal sac is opened twelve hours after introduction of the liquid, the blood-vessels are strikingly stained and contrast markedly with the pallor of the other tissues. The property of absorbing solutions is equally characteristic of arteries and veins of all calibres, and is easily demonstrated by preparing teased specimens of the fresh tissue. The dissolved dye seems to reach the circulation by passing directly through the cytoplasm of the elements of the vascular wall, as well as between the cells.

The omentum is practically undeveloped in pisces, and can play no rôle in the absorption of fluids from the peritoneal area as it does in higher vertebrates. From the study of smears, made at frequent intervals from the surface of the mesentery and peritoneal lining, it would appear that phagocytes play but little part in the primary peritoneal absorption of such colloids as trypan-blue, although a gradual concentration of the dye in large subperitoneal clasmatocytes takes place.

Inside of a few hours all of the body tissues are colored a pale blue, with but few important exceptions. The central nervous system never exhibits a blue coloration; nor does the fat, which is especially abundant in the mesentery of fish, show any staining.

Sections made of the tissues during the first two days reveal practically nothing except a diffuse blue staining, which in some instances is hardly visible under the microscope. Storage of the dye is somewhat more delayed than in mammalian tissue and its presence in definite cells is scarcely to be detected before the third day. Thenceforward its segregation to particular groups of cells becomes more and more striking, till, on the fifth or sixth day, the inclusion of the dye in the mononuclear phagocytes of the tissues is complete.

Specimens, in which the concentration of the dye had attained its greatest intensity, were fixed for the preparation of permanent sections. The tissues, after fixation in 10 per cent formalin, were dehydrated and cleared, imbedded in paraffin and cut at 5 microns. The sections were stained with Meyer's alum-carmin.

The liver, which, in Cyprinidae, is a relatively small organ, is completely concealed by the intestines on opening the peritoneal cavity. It consists of numerous loosely connected lobes, closely adherent to the portal veins and very friable, and in an animal which has been colored vitally, it is the most deeply stained tissue in the body. Microscopically the liver of fish neither presents an arrangement into lobules, as in higher animals, nor does it display the cell-tubes characteristic of amphibia. It consists of strands of cells laid down according to no definite pattern. These are abundantly vascularized from the hepatic portal veins and the hepatic arteries which break up into a number of sinusoid spaces. The sinuses are lined by vitally stained endothelial cells, which in appearance and behavior correspond in every respect to the cells of Kupffer in the higher forms. The vital-dye is densely aggregated in the Kupffer cells, whereas the hepatic cells do not contain particles of stain, although they so often do in other animals. The author is able to affirm, however, that the absence of dye-granules from the hepatic parenchyma is not a fixed rule in fish, since the livers of dog-fish (*Mustelus canis*) which were similarly stained, showed both hepatic and Kupffer cells containing the dye.

The Kupffer cells of ichthyopsida appear to be relatively more numerous than in mammalia; they possess the same power of amoeboid movement and they are frequently seen partially detached from the wall of a sinus or lying perfectly free within one, as round, vitally stained mononuclear cells.

The gall-bladder and bile-ducts show no cellular staining of any description, although demonstrable traces of dye are present in the bile. The color seems to gain access to the bile as a result of direct diffusion through the duct-walls.

The spleen, in the species studied, forms a series of dark red partially connected bodies which are distributed along the entire course of the intestine, and are intimately associated with the lobes of the pancreas. The splenic tissue in Cyprinidae, as in fish generally, belongs to the category of haemolymph-glands. On section, such a nodule reveals a delicate capsule, and a framework of slender trabeculae dipping into the depths of the gland,

which divide it into a labyrinth of spacious vascular sinuses. Two things strike one at once,—the great vascularity of the sinuses, which are densely packed with oval, nucleated red blood corpuscles, and the relatively small amount of spleen pulp, containing but few lymphocytes.

The vascular sinuses are partially lined by endothelial cells which are actively phagocytic towards trypan-blue, which phenomenon allies them with the reticulo-endothelium seen in the mammalian spleen. The reticulo-endothelial tissue in fish is extremely simple, for one meets everywhere only an incomplete layer of vitally stained endothelial cells which line the vascular spaces, whereas more highly developed spleens possess a complicated network of phagocytic reticulum cells. The fact that, one does not find endothelial cells in the act of desquamating, nor free phagocytes within the sinuses as one does in mammals, may be a sign that the fish's spleen is only poorly adapted for phagocytic activity and represents a more primitive stage of development.

The remaining organs of the peritoneal cavity, with the exception of the Wolffian body, may be reviewed quickly, for they showed nothing striking from the standpoint of vital-staining. The pancreas, and the elements of the gonads, both testis and ovary, are practically unstained. The intestines exhibit but a diffuse blue coloring of the serosa and the muscular coats: the contents of the canal are colorless.

The Wolffian body (mesonephros) of the species of Cyprinidae studied, is a flattened, elongated organ, closely applied to the dorsal wall of the coelomic cavity. It stains very deeply with vital-dyes, and presents, on gross inspection, in an animal stained with trypan-blue, a uniform deep blue color. The excretion of the benzidine dyes through the mesonephros commences very quickly after their initial introduction into the coelom.

Trypan-blue, as von Möllendorff ('15) showed by dialysis experiments, is in fact a mixture of two dyes. One of these, a red substance, is very diffusible, the other, a blue coloring matter, passes through semi-permeable membranes only very slowly and under ordinary circumstances masks the red element.

These two constituents are readily demonstrated by putting a drop of trypan-blue upon a piece of filter-paper, whereupon a red zone surrounding a blue center immediately forms. Von Möllendorff actually observed the difference in the rate of diffusion of these two substances in the mammalian kidney by obtaining specimens of urine at frequent intervals. He noticed that the earliest specimens collected were a pink color, whereas the subsequent ones turned to purple and finally to blue.

If a fresh preparation is made, of a bit of tissue from the Wolffian body of a fish eighteen hours after intraperitoneal injection of trypan-blue, the tubular epithelium is seen to be tinged red, while a blue coloration is visible only in the interstitial tissue of the kidney. The assumption is that the tubular epithelium is readily permeable to the pink, but to a slight extent only to the slowly diffusible blue constituent of the dye. After a week or more, the red substance becomes exhausted, the pink coloration of the tubules disappears, and they become colorless, excepting some which exhibit traces of blue. It will be shown, that in the mesonephros of teleosts the diffusion of the blue substance to any extent into the tubular parenchyma and so out into the urine, is prevented by a mechanism presently to be described.

The blood supply of the mesonephros of fish resembles very closely that of the liver. The caudal vein bifurcates close to the cloaca, forming two venous trunks which in turn give off a number of advehent vessels to the Wolffian body. These soon break up into a capillary plexus or rete between the tubules, and finally reunite to open by revehent channels into the posterior cardinal veins. This venous capillary-bed with its afferent and efferent vessels is spoken of as the renal portal system and corresponds in every sense to the hepatic portal system familiar to everyone. The mesonephros derives its arterial blood from branches of the aorta through vessels analogous to the hepatic arteries, on which the glomeruli are formed.

The blue component of trypan-blue with which we are actually dealing in vital-staining, gradually accumulates in the intertubular tissue of the Wolffian body. If at the height of

staining, thin paraffin sections are prepared, one is struck by the remarkable distribution of the accumulated dye. The tubules, as noted in the fresh preparations, are nearly dye-free. The accumulations of trypan-blue in the intertubular substance however, are found in the endothelium of the renal portal system, so that one gazes upon a rich network of brilliantly stained capillaries. The endothelial cells lining them appear to possess a great avidity for benzidine dyes, judging from the enormous concentration of coloring matter within their cytoplasm. The glomerular endothelium, which embryologically is a derivative from the aorta, remains unstained.

The stain occurs also, as has been already noted, as blue, finely granular deposits in the distal cytoplasm of a few of the epithelial cells of the renal tubules, in no way comparable however, to the large masses of dye stored in the adjacent vascular endothelium. The epithelium does not in any way resemble, in its behavior towards vital-dyes, the convoluted tubules of the metanephros, whose living cells are always densely crowded with dye. The cells lining Bowman's capsule are unstained.

It is important to recall that in the liver, where similar vascular conditions prevail, we have a corresponding phagocytic activity developed by endothelium—the Kupffer cells—and that at times the liver parenchyma itself contains vitally stained particles. It seems reasonable to suppose that the phagocytic endothelial cells, observed in the mesonephros of teleosts, are analogous to the Kupffer cells in the liver, and that they possess two or possibly three valuable functions. The first function would be to prevent the excretion and consequent loss to the body, of colloidal metabolites circulating in the blood stream; such a conservation of organic nitrogen would possibly account for the relatively low nitrogen output determined for teleost fish by Denis ('13). The second function might well be a protective one, whereby toxic substances in the circulation, which could easily become injurious to the kidney-parenchyma and impair its functional activity, are rendered innocuous. Such a function would explain the retention of the trypan-blue by the endothelial cells of the renal portal system, and its non-appearance

in the tubular epithelium. Lastly they may be an important factor in the process of reabsorption of substances by the tubules, for, as Bainbridge, Collins and Menzies ('13) have shown in the kidney of the frog, one inorganic substance at least, sodium-chloride, is partially reabsorbed from the glomerular filtrate as it passes down the tubule. The fact that fine dye-granules appear in some of the tubules in spite of the phagocytic activity described for the renal endothelium, might be explained either as the result of reabsorption of trypan-blue from the glomerular dialysate, or, as seems more probable, as an excess which has escaped into the epithelial cells in spite of the barrier provided against particulate matter by the lining endothelium of the renal portal capillaries.

With the exception of the specialized vascular linings seen in the liver, kidney and spleen, the endothelium of the blood-vessels does not stain vitally. The outer coats of both arteries and veins, on the other hand, stain quite deeply, due to the presence of diffuse color in the elastic-tissue of the media and adventitia. For the same reason the bulbous arteriosus appears a dark blue, in striking contrast to the unstained myo- and pericardium of the ventricle. The red blood-corpuscles never exhibit vital-staining, whereas an occasional polymorphonuclear or mononuclear leucocyte in the circulation may show a blue coloration. Such vitally stained mononuclear cells probably escape in small numbers into the systemic circulation from the liver sinuses.

The lymphatic endothelium in Cyprinidae is capable of absorbing and storing vital-dyes, a fact of particular interest which is most easily demonstrated in the lymphatic plexus which arises in the corium of the species studied. If the scales are removed from a vitally stained fish, and a small film of the exposed corium dissected free and placed upon a slide in a drop of glycerin, or stained with alum-carmin and mounted in balsam, and examined, an abundant network of vessels of varying dimensions is visible. Part of these vessels resolve themselves into a lymphatic plexus, whose endothelial lining-cells contain dark blue deposits of granular dye within their cytoplasm, a phenomenon which makes them stand out sharply as

a system of anastomosing channels which are readily distinguishable from blood-vessels. The blood vascular tree contains erythrocytes in many of its branches; one usually finds artery and vein accompanying one another, and it is possible to see blood-vessels crossing the vitally stained canals without anastomosing with them—definite proof of the independence of the two sets of vessels. The blood vascular lining-cells never contain even so much as a trace of vital-dye; and in fact, in fish, trypan-blue has never been observed to occur in the endothelium of any blood-vessels other than the renal and hepatic portal plexuses.

Distally the lymphatics of the corium arborize about the scale-pouches, while proximally they are traceable into large, easily collapsible, thin-walled vessels, whose endothelium is similarly phagocytic towards trypan-blue. These vessels follow a course in the intermuscular septa and finally empty into dorsal and lateral longitudinal collecting channels.

The author has examined preparations from every part of the body in the same way, and has been able to ascertain the presence of lymphatics in nearly all connective tissue. Deeply stained lymphatic sinuses with numerous branches were noticed accompanying the aorta. The course of many heavily stained lymphatics was noted in the septa and membranes of the serous cavities, besides a rich anastomosis of vessels in the subperitoneal tissues. In every instance the lymphatic endothelium shows the same phagocytic behavior towards vital-dyes as that seen in the corium.

The observation that the endothelium of the lymphatic vessels of Cyprinidae may be stained by vital-dyes appears to be of considerable importance. It affords an ideal means, combined with an injection technique such as Allen ('06) used, for studying the distribution of lymphatics in the adult animal, and furthermore it promises to lend itself to a solution of the question of the origin and development of the lymphatic system in fish. Favaro ('06) and Mozejko ('14) feel, from their studies, that the lymphatics of fish arise, by a direct transformation of veins into lymphatic vessels, at a late stage of development; so that they constitute modified veins rather than true lymphatics, in

the sense of outgrowths from the embryonic veins, as in higher vertebrates. McClure ('14) on the other hand believes that he has demonstrated the formation of lymphatics in trout embryos quite independent of the veins, as a series of separated vesicles whose endothelium is of mesenchymal origin. The author's observations would tend to show that the lymphatics of fish are not modified veins in the sense of Mozejko, for as has been stated above, a fundamental difference exists between the endothelium of vein and lymphatic in their behavior towards vital-dyes, which makes it improbable that a sudden transformation from one into the other should occur.

On the other hand it seems unlikely that lymphatics in fish should form from tissue-spaces by the transformation of mesenchyme cells into endothelium instead of from embryonic veins, because nowhere else in vertebrates have lymphatics been proven to arise in such a manner. Clark ('09) demonstrated that in the tadpole lymphatics grow by a process of budding and sprouting, and that lymphatic endothelium is always formed from preexisting endothelial cells, but never from mesenchyme. The author ('16) confirmed Clark's work and showed further that the endothelium of the lymphatic channels of tadpoles is phagocytic towards vital-dyes, an observation which affords a simple method of distinguishing endothelium from mesenchyme cells. From the present report it is apparent that the lymphatic endothelium of teleosts and of amphibian larvae behave just alike towards benzidine dyes; and it does not seem unreasonable to infer that, not only their morphology, but their growth and development are the same.

A last point of interest is that, in fish and amphibian larvae, there is a primitive lymphatic system whose entire endothelium possesses a function, which, in higher vertebrates, is consigned entirely to the reticulo-endothelial cells of specialized parts of the lymphatic apparatus, the lymph-glands. It is difficult to say what the advantage could be of such a specialization in the distribution of nutritional and phagocytic activity (for the storage of benzidine dyes in the cell may be undoubtedly interpreted in these terms), in higher animals.

The 'wandering cells' or clasmatoocytes of fish need no special description for they resemble in every way those of mammalia, although they are not nearly so numerous, and, in fact, there are very few in the subcutaneous tissue and myotomes. They occur abundantly in the membranes of the serous cavities, where they may be seen as 'adventitial cells' in close proximity to blood-vessels. Smears from the peritoneal fluid also reveal the presence of free macrophages in the body-cavity. The mesothelial cells lining the coelomic sac are, however, non-phagocytic.

The origin of the phagocytic mononuclear cells in the serous cavities is as obscure in fish as it is in higher vertebrates, the supposition being that they are derived from histogenous lymphocytes or clasmatoocytes.

The remaining tissues need little description, though the integument requires brief attention. The scales of *Carrasius auratus* are relatively large. Microscopically they exhibit numerous concentric rings or annuli, transected by eight to twelve radii, which diverge from the focus and end at the periphery of the scale. The focus appears to be formed by the fusion of a number of polygonal plates, between which the sutures are plainly visible. In an animal stained *intra vitam*, with trypan-blue, the dye appears in the scales an hour or so after introduction of the substance, a circumstance which accounts for the rapidity with which the animals assume a dusky blue hue. The distribution of the dye in the scale is very characteristic. The sutures at the focus of the scale are colored, and the radii injected with a deep blue stain from the center to the margin of the scale, the whole presenting the appearance of a network with eight or more blue spokes radiating from it. The annuli are unstained. The inference is, that the scale possesses a system of canals or spaces, in close association with the circulation, through which it is possible for nutritive substances from the blood-stream to reach to all its parts. These spaces, if they are actually in connection with the external surface of the scale, might possibly serve as an excretory apparatus.

The central nervous system is unstained, remaining perfectly white throughout, a contrast to the other tissues of the body,

which, on gross inspection, all present a diffuse blue coloration. The behavior of the nervous tissue coincides exactly with the findings in higher animals.

The author wishes to thank Prof. Caswell Grave for helpful suggestions in carrying on this work.

SUMMARY

Fish are readily stained *intra vitam* by injecting weak solutions of benzidine dyes into the peritoneal cavity. Absorption of the dye from the coelom, by way of the blood-vessels into the circulation, and diffusion throughout the body occurs within a few hours.

Concentration of the dye within the cytoplasmic vacuoles of the 'makrophages' of the tissues occurs only very slowly, so that a striking segregation of coloring matter is not demonstrable before the end of a week.

The endothelium of specialized tracts of the vascular and lymphatic apparatus plays an important rôle in the binding and storage of the dye in teleosts.

It may be observed that benzidine dyes are phagocytised by the endothelium of the hepatic sinuses, analogous to the Kupffer-cells in mammals, and by the reticulo-endothelium of the spleen, which however is much less extensive in character in fish than in higher forms.

In addition to the appearance of dye in the liver and spleen, one notices a storage of vital-stain in the endothelium of the renal portal system, a phenomenon which has no counterpart in the metanephros, nor does it occur in the Wolffian body of amphibia.

There is furthermore a widespread storage of vital-dye on the part of the endothelium of the lymphatic vessels, a condition also noted in amphibian larvae, but never known to occur in mammals.

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REACTIONS OF BLOOD- AND TISSUE CELLS TO ACID COLLOIDAL DYES UNDER EXPERIMENTAL CONDITIONS¹

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FOUR FIGURES (ONE PLATE)

In recent years the employment of various colloidal suspensions as a method of intra vitam 'staining' has come into such general usage that it is hardly necessary to give a general review of the literature concerned or to describe the details of the technique. Collargol, lithium carmine, and the colloidal acid azo dyes Trypanblau, Pyrrholblau, etc., are the substances which have been most commonly used, with little or no variation in the final results. Since these substances are practically non-toxic for mammals it is possible to inject the same animal with repeated large doses of aqueous suspensions before he shows any ill effects from the treatment.

The dyes usually appear in the cells in the form of granules, diffuse staining of the cytoplasm and nucleus probably always being an indication of injury of the cell. Since the dyes which have generally been employed diffuse rapidly through the tissues, it makes little difference whether the animal has been stained by intravenous, intraperitoneal or subcutaneous injections. The first two methods are preferred, because tissue necrosis at the site of injection is apt to result from repeated subcutaneous injections unless the parts are carefully massaged and kneaded.

Evans and Schulemann have shown that the rate of diffusion depends on the size of the particles of the dye (acid azo dyes) when in aqueous suspension. The more rapid diffusibility and

¹ Aided by a grant from the Research Funds of the University of Minnesota.

the lessened danger from embolism would, therefore, make the dyes having the smaller particles the more favorable ones for experimental purposes. The rate of diffusion of a given dye also varies somewhat with the different species of animals used, the writer having found that Pyrrholblau and lithium carmine will diffuse much more rapidly through the tissues of a wild rat than through those of a rabbit or guinea pig following subcutaneous injections of the dyes.

The 'dye granules', as they have been termed by Evans and Schulemann, have been reported in many different types of cells belonging to many different tissues, and, as is to be expected, the character of the granules varies considerably in the different types of cells. It is possible that some of these granules may represent instances of true vital staining of preformed structures, as has been claimed for all of the dye granules by several authors, and that others are mere aggregations of dye particles.

Schulemann ('12) believed that the dye granules represent a combination of dye with a reaction body of the cell, and also that various preformed structures, such as plasmosomes, secretory granules, etc., can be made visible by means of the dyes. V. Möllendorf, Pappenheim and Tschaschin also agree with this earlier view of Schulemann. According to Tschaschin the fine granules and rods seen in fibroblasts after Pyrrholblau injection, and the small blue granules of clasmotocytes are chondriosomes, while the larger round granules are secretory granules derived from the chondriosomes. K. J. Scott, however, has shown that the chondriosomes of these cells may be stained with Janus green while the cells contain the dye granules. She could find no relation between the latter and the chondriosomes.

At present Schulemann and Evans believe that the dye granules are merely aggregations of dye particles which have been taken up by a process similar to phagocytosis and deposited within cytoplasmic vacuoles without combining with any constituents of the cell. In other words, the formation of dye granules is a physical process, and not a chemical union of preformed structures or receptors of the cell with the dye. In his

latest paper Schulemann ('16) claims to have demonstrated the physical nature of the granules experimentally.

Although dye granules may be found in many cells belonging to very different tissues, there is one group of closely related cells which shows special avidity for the dyes. With repeated injections these cells will store more and more of the dye in the form of very coarse irregular 'dye granules'. Cells belonging to this group are the so-called clasmatocytes or resting wandering cells of the loose connective tissue, the fixed cells of the reticulum of the hematopoietic organs, especially those which line the sinuses of lymph nodes, and the free cells derived from the reticulum, the v. Kupffer 'stellate' cells of the liver, and the non-granular lymphoid cells of the milky patches of the omentum. The free lymphoid cells of the peritoneal cavity also seem to belong in the group, since many of them show the same reaction after intraperitoneal injections of the dyes. Numerous histological studies by Maximow, Weidenreich, Pappenheim, Downey, and many others have established the intimate relationships between the different members of the group, and to a more or less extent these relationships are now generally recognized, so it is hardly necessary to undertake the extensive review of the literature which would be required in order to point out the details.

Various names have been applied to these cells by those who have studied them by means of the colloidal dyes. They are the pyrrhol cells of Goldmann, the histiocytes of Aschoff-Kiyono, the resting wandering cells of Tschaschin, and the macrophages of Evans. The grouping of all these different types of cells under one term indicates the general belief in their close relationship. Because they are the cells which are especially concerned in the reactions to the colloidal dyes it has been assumed by most workers in this field that the reaction is absolutely specific, i.e., that it always enables us to distinguish clearly between the members of this group and other cells which do not belong to the group, more especially those of the blood.

That the lymphoid cells of various types should be divided into a group which is made up of cells which are primarily blood

cells, and into another group which is of tissue origin, has been claimed by many, particularly by Pappenheim. This idea of the dual nature of the lymphocytes has even been carried over to the lymphoid organs. Here it is supposed that the cells of the spleen pulp and of the interfollicular tissue of the lymph nodes are different in origin and developmental potentialities from those of the follicles. The fact that many anatomists have demonstrated the radial migration of the lymphocytes into the pulp and interfollicular tissue has been completely ignored by those who have accepted this theory.

One of the chief difficulties which the above theory has encountered has been the fact that it is impossible to demonstrate any morphological differences between the lymphoid cells of the tissues and those of the blood and blood-forming organs. However, the solution of this difficulty seemed accomplished with the advent of the methods of vital staining with colloidal dyes, for it was found that intravenous injections of large quantities of the dyes did not result in the staining of any of the blood cells, while all of the cells of the tissues belonging to the group of histiocytes were intensely stained. This led Aschoff-Kiyono, Pappenheim, Schulemann-Evans and others to the belief that the 'dye cells' were always of tissue origin, and this in spite of the fact that Kiyono, one of the most ardent defenders of the specificity of the reaction, was forced to admit that lymphocytes of lymph nodes in the later stages of aseptic inflammation may enlarge and develop relatively more protoplasm, when it is impossible to distinguish them from the true histiocytes of the organ, and impossible to show that they do not take up the dye. Kiyono also admits that in the *tâches laiteuses* of the omentum of vitally stained animals it is difficult to distinguish between the smaller 'histiocytes' containing few granules and the larger lymphocytes. But in spite of this admission of the occurrence of intermediate forms Kiyono concludes that the method is a reliable one, and that the lymphoid cells of the tissues which take up the dye belong to the group of histiocytes rather than to the lymphocytes. To what extent this opinion has gained ground is shown by the recent publications of H. M. Evans and

Frank A. Evans. The latter, in an experimental study of the mononuclears of the blood and tissues, divides these cells into myeloid cells which show the oxydase reaction, macrophages which contain carmine granules, and lymphocytes which do not respond to either of these reactions. The supposed specificity of these two reactions is, therefore, accepted and the lymphoid cells are consequently divided into three distinct groups. According to this classification the bulk of the free cells of the spleen pulp are 'myeloid' cells, and the so-called transitional cells of the blood are also of myeloid origin. The cells with dye granules belong to the group of macrophages which are of tissue origin, while the free wandering cells without dye granules belong to the series of lymphocytes which are derived from the parenchyme of the lymphoid organs.

While the theory of the absolute independence of the histiocytes has been the commonly accepted one it has not passed without opposition. For theoretical reasons it is opposed by Pappenheim ('13) and by Emmel ('16, p. 106-108), both believing that the ability to take up the colloidal dyes is dependent on the stage of differentiation of the cells rather than upon complete genetic independence from other cells which do not ingest the dyes. Pappenheim is inclined to the belief that the histiocytes may be the mother cells of non-granular lymphoid cells which have lost the ability to take up the dyes during their further differentiation. Tschaschin, on the other hand, concludes from extensive studies with experimental inflammation of the loose connective tissue of vitally stained animals, that lymphocytes leave the vessels in great numbers and increase rapidly in size to form 'polyblasts' which take up the dye and store it in the form of the typical dye granules of the histiocytes. From this he reasons that there is no fundamental distinction between histiocytes and lymphocytes, but that the latter must leave the blood or lymph stream before they can take up the dye. Those of the hematopoietic organs or blood and lymph stream are too young and not sufficiently differentiated, or they are not in the proper medium, so they must leave the vessels before they are able to perform their special functions. According to Tschas-

chin then, blood cells and wandering cells of the connective tissue are closely related cells, and in many cases they are the same cells under different conditions and in different stages of differentiation.

Maximow arrived at similar conclusions from a study of tissue cultures of lymph nodes from young and adult rabbits. In such cultures he found that lymphocytes migrate into the plasma where they survive three or four transplantations. The addition of tissue extracts to the plasma prolongs the life of the lymphocytes and enables them to differentiate into typical polyblasts which will take up Trypanblau from the plasma and store it in the form of dye granules. Later these polyblasts cannot be distinguished from those which come from the reticulum. All the intermediate stages are traced and figured, and there seems to be no doubt in this case but what many of the cells which ingested the dye were derived from true lymphocytes of the lymph nodes. This is in harmony with Kiyono's observation that in the later stages of aseptic inflammation of lymph nodes it is impossible to distinguish between the larger lymphocytes and the smaller histiocytes. Kiyono, however, doubts the actual transition from one cell-form to the other.

It is generally conceded that under pathologic conditions, and following the stimulation resulting from repeated injections of the colloidal dyes, the reticular histiocytes from the hematopoietic organs, particularly the spleen, and the stellate cells from the liver may pass into the venous blood where they appear as large mononuclear cells loaded with dye granules. Few, however, are willing to follow Aschoff-Kiyono to the extent of believing that histiocytes form a small but constant percentage of the large mononuclears of normal blood. Although admitting the presence of histiocytes in normal blood Kiyono draws a sharp line between them and the lymphocytes and ordinary mononuclears without dye granules. The histiocytes are derived from fixed 'histioblasts' of the tissues and hematopoietic organs and their number is increased by proliferation of their own kind, while the lymphocytes are derived from the lymphoid parenchyme of the lymphoid organs.

The histiocytes do not take up the dye directly from the circulation but become loaded with it while they are still within the tissues, and their passage into the blood stream is more or less accidental, and is determined to a large extent by the conditions of the experiment. They are difficult to find in blood smears, but, as Aschoff and Kiyono have demonstrated, if the splenic, hepatic, or portal vein be tied off, fixed, embedded and sectioned they may be found in relatively large numbers. The fact that this procedure is necessary might be regarded as favoring the view of Mitamura and Masanori that the anesthetic, or the manipulation during the operation, or premortal agony cause the separation of the endothelial histiocytes from the hematopoietic organs and their entrance into the blood stream. These authors find the histiocytes to be very rare in the veins of living animals, but quite numerous in dead animals.

The view that their occurrence in the blood stream is more or less accidental is strengthened further by the observation of Aschoff-Kiyono that they are rapidly filtered out of the circulation by the capillaries of the lung, the vascular loops of the kidney glomeruli, etc. This shows that the dye cells act as foreign bodies when they reach the blood stream, and it also accounts for the small numbers of histiocytes in the general circulation.

Without further consideration the conclusion that the reaction to colloidal suspensions is a specific one might seem justified. However, Evans and Schulemann have shown that the process of taking up the dyes is really one of ingestion or phagocytosis rather than of true vital staining. If this is true we should naturally expect the reaction to conform to the general laws of phagocytosis. A few references to the literature will be sufficient to indicate a few facts regarding phagocytosis which are of importance in this problem.

While it is true that under certain conditions phagocytosis may take place within the general circulation this is not the rule, as can readily be proven by a few experiments with intravenous injections of foreign matter of various kinds. Recently Rosenthal has again shown that even living organisms are rarely phagocytosed within the circulating blood. Nonvirulent bacteria in-

jected into the tail vein of mouse were phagocytosed by 'endothelial' cells, chiefly the stellate cells of the liver. Wandering cells of the blood became active only when the bacteria were so numerous that the endothelial cells could no longer take care of them. Werigo found anthrax bacilli to disappear from the circulation within a few minutes. They were held by phagocytes in the spleen, liver and lungs. Adami reports similar reactions with various pathogenic bacteria. Bull, studying the fate of typhoid bacilli when injected intravenously into normal rabbits, found that the bacilli were phagocytosed by polymorphonuclears which had accumulated in the capillaries of the lungs, liver and spleen. There was no phagocytosis of bacilli in the blood. In discussing this point he states:

We have found no indications that phagocytosis of the bacteria studied by us takes place in the blood or on a grand scale in the unagglutinated state. Hence, as the bacilli cannot be agglutinated and removed by the organs and also cannot be phagocytosed in the blood stream, they continue to circulate, under some conditions, until they are removed and destroyed by the phagocytes.

Bull also studied the blood of rabbits in which bacteremia had been allowed to develop for ten hours after the injection of virulent pneumococci. The injection of immune serum was followed by immediate clumping and removal from the circulation of diplococci by the lungs, liver and spleen. In this case the organisms were also phagocytosed by polymorphonuclears which had accumulated in the capillaries, sinusoids and blood spaces of these organs. "The act of phagocytosis follows quickly upon the agglutination and removal and, it would appear, never takes place in the blood stream itself; for in the study of hundreds of blood films from the heart only one leucocyte containing diplococci was encountered."

In Rosenthal's experiments only a few of the polymorphonuclears of the capillaries and blood spaces of the organs took part in the process of ingestion, the reticular and endothelial cells being the most active phagocytes. Wyssokowitsch ('86), who was the first to make a detailed study of experimental bacteremia, found *Micrococcus tetragenous* and the typhus

bacillus to be phagocytosed exclusively by the endothelial and reticular cells of those organs in which the velocity of the blood current is greatly reduced, i.e., of the spleen, bone marrow, lungs and kidneys. He used various kinds of organisms, but never found the polymorphonuclears to be active, and never found a case of phagocytosis in the general blood stream, although particular attention was given to a study of blood films. Great variation in the time of disappearance of different organisms from the blood stream was noted, from which it was concluded that the nature of the substances given off by the organisms was of great importance in determining the phagocytic activity of the cells of the organs.

These experiments, and those of Bull, Rosenthal, Metchnikoff and others show that when living organisms are injected into the blood stream they are removed either by the cells lining the blood channels of certain organs or by leucocytes which have accumulated in those organs, but never by the leucocytes in the general circulation. The type of cell involved in phagocytosis seems to depend altogether on the organism used, and a certain amount of isolation from the blood stream is necessary before phagocytosis can take place.

Experiments to be described below show that the type of phagocytic cells involved in the process of taking up unorganized foreign matter depends altogether on the conditions of the experiment. According to Buxton and Torrey this is also true when living organisms are used. Typhoid bacilli and staphylococci injected into the peritoneal cavity of rabbits appeared within macrophages of the mediastinal lymph nodes within one hour. The authors conclude that the macrophages have seized the organisms because they were present before the arrival of the polymorphonuclears. Later the latter invaded the nodes in great numbers and seized whatever organisms were left.

The power to phagocytose may vary greatly in cells of the same type (Hektoen, Rosenow), and cells which are not ordinarily phagocytic may become so under certain conditions, as shown by Achard, Raymond and Foix, who reported a case in which the eosinophils of a pleural exudate were very active as phago-

cytes. Phagocytic eosinophil leucocytes have also been reported by Lattan-Larrier and Parvu, Wendenburg, and by Weinberg-Séguin.

From the above it is evident that phagocytosis is a physiological process which is not confined to any one type of cell, and that the material to be phagocytosed which under ordinary conditions is taken up by a certain type of cell may, under slightly different conditions, be taken up by cells which genetically and structurally are quite distinct from the first type. As examples from Pathology may be cited the well known 'Herzfehlerzellen'—epithelial cells from the alveoli of the lung which have phagocytosed red corpuscles, the neuroglia cells of the nervous system which may become very active phagocytes, and even the ganglion cells of the nervous system which may phagocytose leucocytes when inflammation of the meninges is accompanied by pus formation. Many other examples of this type of pathologic phagocytosis may be gleaned from any good text book of Pathology.

If vital staining with the colloidal dyes is merely a process of ingestion (Evans-Schulemann) then we should expect to find the same variations in the reactions to the dyes that we find with phagocytosis of other kinds of foreign material. Rosenthal and others have shown that phagocytosis of foreign material does not take place within the general circulation under ordinary conditions unless the blood is flooded with so much of this material that it cannot be eliminated within a relatively short period of time, and this in spite of the fact that the blood contains plenty of cells which are known to be active phagocytes. As is to be expected, the same results are obtained when the colloidal dyes are used as the foreign material. There is no ingestion of the dye on the part of cells already present in the blood stream, but it is taken up very quickly by the reticulo-endothelial cells of the liver, lymph nodes, spleen and bone marrow, and by clasmatoocytes, etc., after the stain has diffused into the tissues. The only difference between this reaction and that with which we are familiar when other kinds of nontoxic material are injected is the fact that the colloidal dyes which

are commonly used diffuse readily into the tissues, resulting in a more active phagocytosis of their particles on the part of tissue cells than is the case when less diffusible substances are used. Living organisms are disposed of by the reticular and endothelial cells and so do not get a chance to reach the clasmatocytes and other phagocytic cells of the tissues.

The same material injected into the tissues may cause active migration of the phagocytic cells from the blood stream to the site of injection, where they will soon begin to ingest the foreign substance. This well known fact evidently indicates that conditions in the circulating blood stream are not favorable for phagocytosis, and it also shows that cells which will not take up foreign material from the blood stream will readily do so after they have migrated to the tissues.

Different types of phagocytic cells show a decided preference for certain kinds of foreign matter, and for this reason Metchnikoff divided them into two groups of 'microphages' and 'macrophages.' We now know that one group may act as a substitute for the other if it happens to be the first in the field, and we also know that cells which ordinarily are not phagocytic may become so under certain conditions (phagocytic eosinophils, etc.).

Exactly the same variations are seen when we use the azo dyes as the material to be phagocytosed. The results depend altogether on the conditions of the experiments. No cells of the circulating blood will be stained when the dyes are injected intravenously, but tissue cells, both fixed and free, are seen to be loaded with the dye granules. Very few of the free cells of the peritoneal cavity will contain the dye, but if the injection is made directly into the peritoneal cavity most of its free cells will contain the granules. This difference in the behavior of the peritoneal cells, depending on the method of injection, has already been pointed out by Pappenheim and by Tschaschin. It is also seen that more of the cells of the milky patches of the omentum will contain the dye following intraperitoneal injection than is the case when the dye has been injected into the veins. With subcutaneous injections of the dye we find that

most of it is contained in large cells of the macrophage or polyblast type which are presumably all of tissue origin. However, we also find plenty of smaller cells with all the morphological characters of lymphocytes which also contain the dye in the form of granules. Aschoff-Kiyono, Schulemann-Evans and others count all of these cells with the group of 'histiocytes' or 'macrophages,' thus indicating their belief that these cells are always of tissue origin. They are supposed to have no immediate genetic relationships with lymphocytes which might have migrated from the blood vessels. However, with the work of Tschaschin, and the studies of Maximow with tissue cultures of lymph nodes, together with the variations in the reported behavior of the free cells of the peritoneal cavity, depending on the method of injection, before us we are not so sure that lymphocytes are not concerned in the reaction. It is quite possible that many of the large polyblasts of the subcutaneous tissue are enlarged phagocytic lymphocytes which have migrated from the vessels.

The above experiments indicate that under the special conditions with which they were carried out the dye is preferred by the cells which Metchnikoff included in his group of macrophages, and also by all of those cells, both fixed and free, which H. M. Evans has included in the group. The experiments do not prove, however, that this group of cells is entirely separate from and genetically independent of the lymphocytes from the parenchyme of the lymphoid organs and the blood stream. On the contrary, we already have many observations which seem to indicate that the lymphocytes from the blood should be included in the group. The fact that these lymphocytes will not take up the Pyrrholblau while they are still in the circulation is of no significance, for we know that the polymorphonuclears which are active phagocytes for most kinds of bacteria and for other foreign matter will not become active until they have reached the tissues.

It is known that the polymorphonuclears may phagocytose material which under ordinary conditions is taken up by the large mononuclear macrophages, as, for example, in the experi-

ments of Schott with the injection of foreign erythrocytes into the peritoneal cavity, which resulted in the phagocytosis on the part of a few of the polymorphonuclears present of the erythrocytes and their fragments. If vital staining with the azo dyes is a matter of phagocytosis rather than of true staining of pre-formed structures it should be possible to induce the polymorphonuclears to take up the dye under similar conditions. The other phagocytic cells of the blood, the large mononuclears and larger lymphocytes, should also phagocytose the dye under the same conditions.

The loose subcutaneous tissue is not a very favorable tissue for such experiments, because it contains so many clasmatocytes and wandering cells of other types which proliferate rapidly after the injection of the dye that the cells which migrate from the blood vessels get little chance at it. Polymorphonuclears are numerous within five hours after the injection, but none of them contain dye granules, because the clasmatocytes and polyblasts present have already taken up the dye. This is probably due to the fact that the dye diffuses rapidly over a comparatively wide area, bringing many macrophages which are already present in the tissue in contact with it.

If a less diffusible substance, such as ordinary carmine ground up in salt solution, is used the results are at first very different. The salt solution filters out through the surrounding tissue, leaving the carmine granules in a dense mass at the point of injection. This mass is invaded by polymorphonuclears which rapidly phagocytose the granules. However, even in this case, those macrophages at the edge of the mass which come in contact with the carmine show a greater avidity for it than do the polymorphonuclears. The lymphoid mononuclears gradually increase in number and invade the mass of carmine. The carmine spreads, probably largely through the agency of the polymorphonuclears which drag it into the surrounding tissue. The polymorphonuclears degenerate, at the same time liberating the carmine. This is taken up by the macrophages which, in the mean time, have become very numerous. In the later stages all

of the carmine is contained in the lymphoid mononuclear cells and all of the polymorphonuclears have disappeared.

In the peritoneal cavity Pappenheim-Fukushi report the presence of carmine granules in polymorphonuclears following the intraperitoneal injection of lithium carmine. This seems to be rather exceptional for the peritoneal cavity, probably because of the normal presence of numerous cells belonging to the macrophage series.

The intramuscular connective tissue contains very few clasmatoocytes and free lymphoid wandering cells, and hence when the dye is injected there it is not disposed of as rapidly as in the subcutaneous tissue. The density of the tissue prevents rapid diffusion. The result is, that when the polymorphonuclears appear on the scene there is still plenty of free dye present. The leucocytes being thus in direct contact with the dye are able to phagocytose it.

The polymorphonuclears store the dye in exactly the same form as it is stored in the macrophages or histiocytes. It is concentrated in the form of large, irregular blue granules identical in appearance with the dye granules of the macrophages of the subcutaneous tissue or of the reticulo-endothelial cells of lymph nodes. Polymorphonuclears with dye granules from the intramuscular tissue of a white rat are shown in figure 3. That the 'dye granules' are not the specific granules which are seen when a blood smear is stained with an ordinary blood stain is shown by a comparison of the dye granules of figure 3 with the granules of the polymorphonuclears from the circulating blood of the same animal shown in figure 1. Because it has become flattened during the process of making the smear the latter cell appears much larger than the cells from the section of the intramuscular tissue. Nevertheless, its granules are evidently much smaller than are the dye granules of the more compact cells from the sections of the muscle.

The muscle sections contain a few cells having the general characters of medium-sized and larger lymphocytes, excepting that they contain typical dye granules. According to Aschoff-Kiyono they are therefore 'histiocytes' of tissue origin, and not

lymphocytes. Their number is so small that it is impossible to determine from this experiment whether or not they have migrated from the vessels. The next experiment, however, shows that they might be true lymphocytes from the blood stream.

This experiment consisted of the injection of Pyrrholblau into the doubly ligatured femoral vein of a white rat. The ligatures were placed first, and the dye was then injected into the lumen of the vessel between the two ligatures. The next day the isolated segment was removed, fixed in Helly's Zenker-formol mixture, imbedded and sectioned. Figure 4 is a drawing of a low power view of a portion of one of the sections. All of the polymorphonuclears which came in contact with the dye are loaded with it, and it is concentrated in the form of the typical 'dye granules.' There is no diffuse staining of cytoplasm or nucleus, which indicates that the leucocytes were living at the time of fixation. The nuclear stain was obtained by counterstaining the sections with hemalum. Without a counterstain the nuclei are absolutely colorless, while the blue granules are very conspicuous. The granules are identical with those of the polymorphonuclears of the sections of muscle.

The low power drawing (fig. 4) gives a good idea of the large number of polymorphonuclears present in the vessel. It is quite evident that many of them have migrated in from the surrounding tissue or from the vasa vasorum. There has apparently been no increase in the number of lymphocytes, for they are not more numerous than would be expected in the amount of blood included in a section through the vein.

The large and medium-sized lymphocytes in the vessel also contain the dye granules, as is seen in figure 2, and a few small lymphocytes were found which contained one or two dye granules. This seems to dispose definitely of the idea that lymphocytes cannot take up the dye granules, for in this case we are not dealing with 'histiocytes' from the tissues.

This and the previous experiment have shown clearly that the polymorphonuclears, cells which are undoubtedly blood cells, may take up the colloidal dyes when they are isolated from the general circulation, and the experiment with the ligatured vessel

proves that lymphocytes which are already present in the blood will also take up the dye when a condition of stasis is brought about by the application of the ligatures.

These results are exactly what might be expected when they are considered in the light of what is known about phagocytosis in general. For example, in bacteremia it seems that phagocytosis of the bacteria in the general circulation is of rare occurrence, but in the capillaries of the lung, and liver, and blood spaces of the spleen and bone marrow the process may be very active. Here the organisms are disposed of by the leucocytes or by the endothelial and reticular cells. In these situations the blood current is slowed down, and the conditions of the ligatured vessel are to a certain extent duplicated.

Experimental pneumonia in animals stained *intra vitam* with Trypanblau, as reported by Kline and Winternitz, is of interest in this connection. Polymorphonuclears containing dye granules were found in the alveoli, bronchioles and blood vessels of the involved lung but not in the general circulation. Injection of the blood vessels of the involved lung showed that they were cut off from the general circulation by plugs of fibrin in the capillaries.

These facts show clearly that blood cells, polymorphonuclear leucocytes as well as lymphocytes, will take up the colloidal dyes and store them in the form of coarse 'dye granules' whenever the cells become isolated from the general circulation. The cells may leave the blood vessels and phagocytose the dye in the tissues, or they may take it up while still within the vessels in case the latter have become isolated from the general circulation. The reaction to the colloidal dyes, therefore, does not differ from that which results from the injection of other foreign material including nonvirulent living organisms. Slight differences in detail due to the diffusibility of the substances injected, etc., are of no significance.

It is evident that the reaction to the colloidal dyes is no more specific than is general phagocytosis. It is true that a given type of phagocytic cell may show preference for a certain kind of foreign material, but under slightly different conditions the

same material may be taken up by cells of a different type. It has been shown that the same is true when the azo dye Pyrrholblau is used as the foreign material. The reactions of cells to this dye are, therefore, not sufficiently specific for determination of genetic relationships.

The fact that lymphocytes will take up the dye from the ligatured vessel shows that many of the wandering cells of the connective tissue may be lymphocytes which have come from the vessels. The presence of dye granules in a wandering cell of the connective tissue is no proof for its histogenous origin. It may be one of the larger lymphocytes which has migrated from the vessels and taken up the dye without further differentiation, or it may be a lymphocyte which has differentiated into a 'polyblast' (Maximow, Tschaschin).

The lymphocytes containing dye granules are usually the larger lymphocytes and large mononuclears which do not seem to have undergone any further differentiation into 'polyblasts.' This is contrary to the findings of Maximow in tissue cultures from lymph nodes, and it seems to indicate that the lymphocytes of the blood are more mature than are those of the nodes. Tschaschin concluded that they must leave the vessels before they are capable of taking up the colloidal dye. This evidently is not the case, but isolation from the general blood stream is necessary. The special conditions of the tissues are duplicated in isolated vessels, at least in so far as phagocytosis is concerned.

In the lymphoid organs the lymphocytes seem to be too immature to be able to act as phagocytes. In the tissue cultures (Maximow) they are capable of further differentiation and of phagocytosis, but it is doubtful whether this is possible in the lymph nodes under ordinary conditions. According to Kiyono they may undergo further differentiation in the later stages of aseptic inflammation and it is then impossible to distinguish the larger ones from histiocytes (Kiyono). Babkina states that they may differentiate into 'polyblasts' under these conditions, and Maximow has found these polyblasts to take up colloidal dyes in tissue cultures.

The chemical and mechanical conditions of the blood stream are not favorable for phagocytosis which has been noted here only under exceptional conditions. The same conditions which prevent mitosis of lymphocytes in the circulating normal blood may also inhibit phagocytosis and prevent further differentiation of these cells as long as they are in the blood stream. In the thoracic duct mitotic figures are numerous and many of the cells contain ingested erythrocytes, etc., but when they reach the blood stream their phagocytic and proliferative activity is temporarily suspended. The constant churning action to which the cells are subjected in the general circulation may also have the effect of slowing up any phagocytic activities on their part. Under these conditions the cells are probably not in contact with the foreign particles for sufficient length of time to be able to phagocytose them. This would be especially true of substances like the azo dyes, which are eliminated from the circulation very quickly. There is more chance for phagocytosis if the substance remains in the circulation for a long period of time, as is the case when carmine is injected. Hoffmann and Langerhans report the presence of carmine as late as 148 days after injection. They could find none of it in the lymph nodes until three days after the injection, which indicates that this substance is eliminated from the circulation very slowly. In the blood it is taken up by the "ordinary white corpuscles."

Although it is difficult to determine the exact conditions which influence phagocytosis in the blood stream, it seems likely that the time during which the foreign substance remains in the circulation, as well as chemical and physical conditions, is of importance. Isolation of a segment of a vessel from the general circulation, or migration of the leucocytes and lymphocytes from the vessels places these cells under such favorable conditions that they are soon able to begin their special activity as phagocytes.

Both lymphocytes and polymorphonuclears take up Pyrrholblau from a ligatured vessel, and the intramuscular tissue also contains cells of both types with dye granules. We know that polymorphonuclears of this location have come from the vessels,

but it is difficult to prove that any of the lymphoid cells with dye granules have also migrated from the vessels. However, since the lymphocytes of the ligatured vessel take up the dye very freely it is more than likely that many of those which migrate to the tissues will also phagocytose the dye. The larger ones can probably take it up immediately, but the smaller ones would naturally require some time for further growth and differentiation. This would explain the conditions in the *tâches laiteuses* of the omentum in vitally stained animals. The smaller and more immature lymphocytes in the central portion of the nodules take none of the dye, while the larger and more highly differentiated ones in the peripheral regions take it up very freely. Since it has been shown that the lymphocytes of the blood may take up the azo dye there is no longer any reason for separating the peripheral cells as 'histiocytes' from the true lymphocytes. They are all lymphocytes, and in so far as their reaction to the dye is concerned it makes little difference whether they are of tissue origin or whether they have recently migrated from the vessels. Their state of differentiation and the special conditions under which they happen to exist at the time are of chief importance in determining their reaction to the dye.

Full consideration of the results of these experiments adds new and strong evidence in favor of the view of Schulemann-Evans, that vital 'staining' with these dyes is primarily a process of ingestion or phagocytosis, at least in so far as the cells considered here are concerned. If we were dealing with a process of true staining of preformed structures of the cell it would be difficult to account for the variations in the reaction under different experimental conditions. Whether the ingested colloidal material finally combines with constituents of the cell protoplasm is another question and one which can not be settled with experiments of this nature. The impression gained from working with a considerable mass of material is, that the dye granule is more than a mere concentration of dye particles in a cytoplasmic vacuole. The granules become very firm and resistant to reagents; they may be stained, and in other respects their behavior is more like that to be expected of a structural

constituent of the cytoplasm. Phagocytosis, however, seems to be the process which gets the dye into the cytoplasm, and the dye granule which results is certainly not a preformed structure, for such granules cannot be demonstrated with any other methods.

Admission that the process is one of ingestion and storage rather than of true staining forces the giving up of the idea of the specific nature of the reaction, for we have seen that phagocytosis is not a specific reaction confined to any one group of cells. We are, therefore, no nearer to the solution of the problems for which the method has been used than we were before the advent of the azo dyes. The attempt to classify cells according to their reactions to the colloidal dyes must, therefore, be regarded as a failure.

SUMMARY

1. *Intra vitam* 'staining' with the azo dye Pyrrholblau is a process of ingestion and storage, and not one of true staining of preformed structures.

2. Blood cells, both lymphocytes and polymorphonuclears, will ingest and store the dye after they have migrated into the tissues, or when the blood vessel which contains them has been isolated from the general circulation. In this respect the blood cells behave just as they do toward living organisms or other foreign material.

3. The method is not specific and cannot be used for the purpose of distinguishing between cells of tissue origin and those which have come from the blood stream.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Polymorphonuclear leucocyte from blood smear of albino rat. The granules are the specific granules of these leucocytes, and not dye granules.

2 Dye granules in lymphocytes from section of doubly ligated femoral vein of albino rat.

3 Dye granules in polymorphonuclears from section of rat muscle injected intra vitam with Pyrrholblau.

4 Portion of a section of the contents of doubly ligated femoral vein of rat. Intra vitam injection of Pyrrholblau between the ligatures. Vein removed and fixed twenty hours later. Dye granules in the polymorphonuclears. Dye granules in lymphocytes of this same vessel are shown in figure 2.



Fig 1



Fig 2



Fig 3

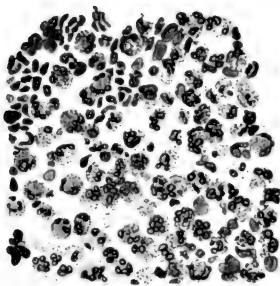


Fig 4

Helen A. Sanborn, del.

OBSERVATIONS ON THE OCCURRENCE OF EOSINOPHILIC LEUCOCYTES AND THE GRANULE CELLS OF PANETH IN THE VERMIFORM APPENDIX OF MAN

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The comparative infrequency of careful descriptions of the occurrence and distribution of many cell types encountered in the vermiform appendix of man, has led us to make a study of this organ. This is all the more important, since an accurate knowledge of the normal cellular content of this organ is essential in making a study of many of the less definite pathological reactions which occur in the appendix. This paper embodies the results of a careful analysis of specimens of normal appendices with regard to the presence of Paneth's cells and the normal content of eosinophilic leucocytes. These specimens have been obtained as a result of the routine removal of this organ in instances of laparotomy for abdominal and pelvic lesions other than those of the appendix. Only those specimens have been selected that are free from angulations, bands of adhesion, dilations, obliterations, lymphoid destruction, parasitic worms, and inflammatory exudates.

EOSINOPHILIC LEUCOCYTES

Although it is generally recognized that eosinophiles are normally found in the mucous membrane of the alimentary tract, there are only a few references to the number, distribution, and frequency of the occurrence of these cells in the vermiform appendix. According to Schwarz ('14), Oehler ('12) found eosinophiles very frequently in the vermiform appendix of man; Loele ('11) and Aschoff ('08) also have mentioned the great numbers

found in this organ. Aschoff, moreover, has noted that while the appendix of the newborn contains only a few of these cells, in the suckling there is an increasing number and in the appendix of the adult a great many eosinophiles. For a careful and extensive review of the literature dealing with cells containing eosinophilic granules, the reader is referred to the monograph by Schwarz.

It is not the object of this paper to go into the nature of the eosinophilic granules, or the significance of the presence of these cells in the appendix, but rather to describe the frequency of their occurrence, their distribution and their origin. These observations are based upon the study of sixty normal appendices taken in the consecutive order as they came into the laboratory of pathology.

These specimens were fixed in 10 per cent commercial formalin, 10 per cent formalin neutralized over magnesium carbonate, Zenker's solution and Bensley's fluid. Paraffine sections of 5 microns were prepared. These were stained with hemotoxylin and eosin and with the molybdenum alizarin lac method of Okajima ('16). The Winkler reaction was used as follows for the demonstration of oxidase granules in the eosinophilic leucocytes. After formalin fixation frozen sections and paraffine sections brought down into distilled water, were treated for two minutes with the following freshly prepared mixture: 1 per cent alpha-naphthol in a 1 per cent KOH added to an equal quantity of a 1 per cent aqueous solution of dimethylparaphenyldiamin hydrochloride. They were then washed and mounted in distilled water. All oxidase granules appear a blue-black. Under a 4 mm. objective, camera lucida drawings of the cells in areas reacting positively to the Winkler test were made. Cover glasses were lifted off the sections and the indophenol blue was dissolved by the means of alcohol. Sections were then returned to water, stained with hemotoxylin and eosin and mounted in balsam. By superimposing upon the former drawing, it was possible to identify the cells containing the oxidase granules. These with very few exceptions were eosinophilic leucocytes. It is interesting to note in this connection that in

the study of these specimens and many other tissues, both normal and pathological, we have found that in paraffine sections the eosinophile is the only cell in which granules of indophenol blue can be satisfactorily demonstrated.

The eosinophiles are found in relatively large numbers in the tunica propria and submucosa. With the Winkler reaction, the boundaries of the glands and the lymph nodules are distinctly marked by one or more rows of indophenol blue containing cells which prove to be eosinophiles. Frequently these cells are seen migrating through the epithelium. They have never been observed in this series within the lymphoid nodules. Rarely are they found in the muscular coats, but occasionally are encountered in the serous coat. The vast majority are found free in the tissues but they are sometimes seen within the blood vessels. Eosinophiles were found in every one of these sixty specimens which had been selected on account of their normal character. The majority of these cells present typical indented or polymorphous nuclei although some of them contain spherical nuclei.

While experimenting with an alizarin stain as described by Okajima ('16) it was applied to sections of several of these appendices. This stain is supposed to be elective for hemoglobin. It was found that the granules of the eosinophiles took the stain equally as well as the erythrocytes. This brings up the question again as to whether the eosinophilic granules are of hemoglobinous nature. Either this is the case or the stain is not an elective stain for hemoglobin.

To determine whether the eosinophiles found in the stroma of the appendix are formed in situ or are of myeloid origin, the indophenol blue synthesis, or Winkler reaction, was used as outlined above. It appears definitely established by the work of a number of German investigators that indophenol oxidase granules are found after formalin fixation only in the lachrymal and thyroid epithelium and in cells of myeloid origin. It has been used in this country by Evans ('15, '16 a, b, c, d) upon blood smears and exudations in a study of the mononuclear leucocytes and also in a study of the reaction found in acute

inflammatory tumors of spleen. The reader is referred to the above papers by Evans for a review of the literature. Recently Forman and Warren ('17) have used it as a means of identifying the tumors of myeloid origin. After applying the Winkler reaction upon sections of these appendices, it was found that with the exception of an occasional neutrophilic polymorphonuclear or mononuclear leucocyte every cell containing the indo-phenol blue was an eosinophilic leucocyte. It was also found that the indophenol blue must be dissolved with alcohol before the α -granules would take an eosin stain. It would appear, therefore, that the indophenol blue was formed in such a way as to protect the α -granules from subsequent staining. If the Winkler reaction is applied after the eosin, however, it is found that the eosinophilic granules are obscured by the formation of the indophenol blue. The eosinophilic leucocytes free in the tissues react in the same manner as do those that are found in the lumina of the blood vessels. The above findings warrant the conclusion that the eosinophilic cells found normally in the appendix are of myeloid origin.

THE GRANULAR CELLS OF PANETH

Paneth's cells have been described as occurring in a great many of the mammals as well as in some of the lower vertebrates. It is generally recognized that these cells occur at the base of the glands in all portions of the small intestine in man. They, however, have been described by some as occurring in the glands of the stomach. Bloch ('03) has described them in glands of the large intestines of nursing infants. Schmidt ('15) on the other hand, failed to confirm Bloch's observation but did describe them as occurring in the vermiform appendix. He examined 66 specimens and found them in 30 instances. Age appeared to have no influence upon their number. They did vary greatly as to the number present in the several specimens. This is the only reference to the occurrence of the cells of Paneth in the appendix of man with which we are familiar. Klein ('06) observed that, in the opossum, these cells occurred not only in the basal portions of the glands of the small intestine

but also on the sides of the villi. He, further, demonstrated that, in the guinea pig, the granule content of the cells bore a relation to the taking of food.

During the progress of the study of the presence of eosinophiles in the vermiform appendix, the routine fixation methods were changed from 10 per cent commercial formalin and Zenker's solution to 10 per cent formalin neutralized over magnesium carbonate and Bensley's solution made by adding 10 cc. of neutralized formalin instead of glacial acetic acid in the Zenker formula. It was in this way that our attention was called to the presence of Paneth's cells in this organ. A neutral fixative appears essential for a sharp staining of the granules of these cells.

In sections stained in hematoxylin and eosin after a neutral fixative, the granules in the Paneth cells stain a brilliant red. It was found advisable not to draw the hematoxylin with acid as it renders the granules less distinct. A better method of staining, however was the use of iron alum hematoxylin followed by mucicarmine for ten minutes. The granules stained black while the mucin of the goblet cells stained red or pink.

The granular cells of Paneth were found in each of the eleven normal appendices examined after the neutral fixation. The number varied in the different specimens. In some instances only one or two cells were found after examining several sections. Examination of other specimens revealed many of these cells in a single section. Their distribution appeared to be uniform throughout the appendix. Sections made from the distal, middle and proximal portions presented approximately the same number of these granular cells. These cells were uniformly situated in the base of the glands.

The presence and number of these cells in the specimens examined did not appear to bear any relation to age. These findings agree with those of Schmidt except that these cells occur more constantly in our somewhat smaller series. On account of the nature of the material, no attempt has been made to determine whether the number of granules bore any relation to the ingestion of food. It, however, is to be remembered that these specimens were from surgical patients who were fasting.

It is not the purpose of this paper to enter into a discussion of the significance of the presence of the cells which have been shown by Klein to be concerned with secretion in the intestine of the guinea pig and to be increased in this animal during fasting periods.

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AN ANATOMICAL CONSIDERATION OF THE CEREBRO-SPINAL FLUID

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INTRODUCTION

Although an extensive literature dealing with the cerebro-spinal fluid and its meningeal pathways has developed during the last forty years, there still seems evident a lack of a comprehensive understanding of the anatomical relations of these fluid-channels. While it may be unfair to judge the trend of anatomical knowledge and progress by the viewpoints expressed in the text-books in this subject, still the many inaccuracies and disagreements concerning the processes of the cerebro-spinal fluid appearing in the more recent of these publications seem hardly warranted. The chief difficulty in all the writings deals with the relationship and possible connections of the meningeal channels with the true lymphatic system. The whole consideration of the cerebro-spinal spaces as presented in current treatises suggests strongly the anatomy of true coelomic serous cavities. Quite apart from the perhaps pardonable ignorance regarding the anatomical aspects of the circulation and distribution of the cerebro-spinal fluid, is an even greater confusion about many of the problems of these fluid-retaining membranes, due in part at least to a misunderstanding of the physiological requirements of such pathways.

A common cause of error in the discussions of the cerebro-spinal spaces has been the abundant references to the fluid of these spaces as 'lymph.' The earlier usage of the word 'lymph' as meaning almost any body-fluid has largely been done away with in physiological writings and today a distinction should

surely be made between tissue-fluid¹ (extravascular lymph?) and the liquid of lymphatic vessels (lymph). This change in the viewpoint in regard to the more restricted use of the word 'lymph' has been largely brought about by the demonstration that the lymphatic vessels are closed tubes and are not connected directly by any openings to tissue-spaces or serous cavities. While physiologically, an apparently direct communication may be afforded for the passage of fluids into the lymphatics, anatomically the lymphatic system is wholly closed. Not only anatomists but physiologists and pathologists have offended by using the word 'lymph' to refer loosely to the cerebro-spinal fluid as well as to most of the other body-fluids. It may be said that the fluids of the body have thus been grouped together, largely because of ignorance regarding their exact functions and processes.

The cerebro-spinal fluid in its physical and chemical characters, is a fluid wholly different from the lymph of the true lymphatic vessels. Halliburton ('04) described this fluid as being a clear limpid liquid of a low specific gravity (1.004 to 1.006), containing few cells and little protein, with a small salt content and possessing dextrose in definite traces. Very recently, this same investigator ('16), in his presidential address before the Royal Society of Medicine, likened the cerebro-spinal fluid to an ideal physiological salt solution. At the same time, Halliburton advanced arguments for the physiological conception that this fluid subserved a definite function in the nourishing of the nervous tissues, and that only in such a restricted sense could the fluid be called the lymph of the brain. But a close and careful differentiation between the chemistry of this fluid and of the lymph of systemic lymphatic vessels was strongly emphasized by this worker. Mott ('10) in his Oliver Sharpey lectures, and

¹ This term was used by Sabin in her Harvey Lecture on "The method of growth of the lymphatic system" (Harvey Lectures, Series IX, New York, 1915-1916) to designate the fluid of the tissues. The term 'tissue-juice' was not employed as it has so frequently been applied to the fluids obtained by subjecting tissues to great pressures in a Buchner press. The term 'tissue-fluid' seems very appropriate.

Cushing in a more recent paper ('14) have likewise presented excellent conceptions of the fluid from somewhat different aspects.

The importance of the cerebro-spinal fluid in the clinic has been recognized since Quinke demonstrated the ease of obtaining it by lumbar puncture. Pathologically the meninges and the subarachnoid fluid have received a great deal of attention, and since the time of Key and Retzius ('76), many observations have been made upon the physiology of the fluid. But the anatomy of the cerebro-spinal spaces (i.e., the pathways for the cerebro-spinal fluid) has not until recently received its due measure of attention. This avoidance of an anatomical viewpoint in the investigations in this field seems to offer a justification for this article. For it is proposed here to present merely a brief account of the anatomical relationships of the pathways for the cerebro-spinal fluid: to present the cerebro-spaces from the aspect of the functional employment of these channels. While logically this presentation should deal first with the embryology of these fluid-pathways about the nervous system, it seems best to bring up to the present the conception of the adult anatomy of this region and then proceed to the developmental problems of the subject.

THE INTRAVENTRICULAR SOURCE OF THE FLUID AND ITS INTRA-VENTRICULAR COURSE

The first hypothesis regarding the origin of the cerebro-spinal fluid may be referred to Haller (1757) who considered it to be the product of the leptomeninges. This belief in regard to the source of the fluid was also expressed by Magendie ('25). Faivre in 1853 and Luschka in 1855 independently suggested the choroid plexuses of the cerebral ventricles as the elaborators of the fluid. The evidence for this view was for a time solely histological and the hypothesis was not placed on any firmer basis until nearly fifty years afterward. The fact that these villous projections into the ventricles possessed a glandular morphology, however, caused a wide acceptance of this conception. Pathologically, also, the intraventricular origin of the fluid seemed established in those cases of hydrocephalus subsequent

upon obstruction within the aqueduct or other interventricular channel.

More convincing proof of the production of cerebro-spinal fluid by the chorioid plexuses was given by the observations of Cappelletti ('00 a, b) and of Pettit and Girard ('02). The first of these workers was able to increase the rate of flow of fluid from a cannula after the administration of pilocarpine, muscarin and ether. Pettit and Girard described for the first time histological changes in the cells of the plexus indicating an augmented production of fluid. Following these important observations, a number of other observers, particularly Meek ('07), Mott ('10), Pellizzi ('11 a, b) and Hworostuchin ('11) have added histological data in support of this viewpoint. Findley ('99), shortly before the action of drugs was tried, presented an excellent pathological consideration of the plexuses. Dixon and Halliburton ('13) have demonstrated changes in the rate of production of the fluid after injection of certain tissue-extracts and of drugs.

Somewhat more definite proof of the relation of the chorioid plexuses to the elaboration of the cerebro-spinal fluid has been offered by Dandy and Blackfan ('13, '14) who produced hydrocephalus by blocking the aqueduct. Removal of the chorioid plexuses in such an experiment prevented the development of the hydrocephalus. In another way, the writer ('14c) was able to show varying rates of elaboration of the fluid when a catheter was inserted into the third ventricle through the aqueduct, demonstrating that the increased elaboration of fluid was not to be referred to an increased permeability of the cerebral capillaries, and also that the intraventricular production of fluid could be influenced by mechanical (vasomotor) and chemical means (Weed and Cushing ('15)).

From this evidence—histological, pharmacological and physiological—the function of the cells of the chorioid plexuses as the elaborators of a characteristic body-fluid, the cerebro-spinal fluid, seems established. It must be understood, however, that these structures, while undoubtedly producing by far the greatest portion of the cerebro-spinal fluid, constitute merely the in-

traventricular mechanism for fluid-elaboration. There is, also, further production of cerebro-spinal fluid by the nervous tissue itself—a small addendum poured by way of the perivascular channels into the subarachnoid spaces. This mode of fluid-manufacture will be discussed in a later paragraph. Furthermore, a minimal production by the ependymal cells, negligible in its significance and total amount, may occur.

The cerebro-spinal fluid, elaborated by the chorioid plexuses, is poured into the cerebral ventricles, which are lined by smooth ependyma. That portion of the fluid formed in the lateral ventricles escapes into the third ventricle and thence by the aqueduct into the fourth ventricle. Likewise, an ascending current of fluid apparently occurs in the central canal of the spinal cord; this, representing a possible product of the ependyma, may be added to the intraventricular supply. From the fourth ventricle the fluid is poured out into the subarachnoid spaces; there is no evidence that functional communications between the cerebral ventricles and the subarachnoid spaces exist in any region except from the rhombic ventricle.

Some uncertainty as to the exact mode of escape of the cerebro-spinal fluid from the fourth ventricle into the subarachnoid spaces still persists. The weight of evidence surely inclines toward the consideration of the foramen of Magendie as a true functioning communication in the inferior velum. This medial foramen has been termed by many observers an artifact, but developmental observations—those of Hess ('85) particularly and of Blake ('00)—indicate that there is a true anatomical break in the membrane. The findings of Cannieu ('97) and of Wilder ('86, '93) should be included here in support of the actual existence of this medial opening. Blake's conception of the formation—a shearing off at the base of a finger-like evagination of the rhombic roof—receives some support from Wilson's ('06) studies of the calamus region and the nucleus postremus. Likewise Retzius' ('96) description of a constant pial fold constituting the posterior wall of the fenestration, in addition to the variable true obex, adds support to the conception of the actual occurrence of a median foramen. The two lateral foramina,

those of Luschka, connecting the lateral recesses of the fourth ventricle with the subarachnoid spaces, seem to have as actual an existence as the medial opening. It is probably through these three foramina—or surely in the region of the inferior tela chorioidea if through an intact permeable membrane—that the cerebro-spinal fluid, produced in the cerebral ventricles, passes into the subarachnoid spaces.

THE MENINGEAL SPACES

The cerebro-spinal fluid, passing through the foramina (or through permeable membranes) of the rhombic roof, enters the subarachnoid spaces in the medial cerebello-bulbar angle. The immediate reservoir into which the fluid is poured is the cisterna cerebello-medullaris; from this region the further distribution of the cerebro-spinal fluid is accomplished. This new product of the chorioid plexus slowly diffuses downward into the spinal subarachnoid spaces, but passes more rapidly upward about the base of the brain and thence, more slowly, over the hemispheres, covering the whole cerebro-spinal axis in a perimedullary arrangement. The evidence for this extraventricular course of the fluid, as outlined, is based on the rather rapid spread of dye-stuffs throughout the subarachnoid spaces, after their introduction into the cerebral ventricles. Replacements of the embryonic intraventricular fluids likewise give indisputable support for this complete perimedullary arrangement.

In the vertebral canal and in the cranium, the anatomical characters of the subarachnoid spaces are similar. The whole space is usually described as being bounded on the outer side by the inner surface of the arachnoidea and on the inner side by the pia mater. By analogy, then, with the coelomic serous cavities, the arachnoid becomes the parietal layer of the space and the pia the visceral layer. In addition, the space is described as being interrupted somewhat by processes of the arachnoidea which run into the pia. The presence of the ligamenta denticulata in the spinal region does not in any way affect the essential anatomic relations of the space.

But such a method of description hardly takes full cognizance of the essential character of the arachnoidea and of the pia mater as constituting merely limiting structures for an intra-membranous series of channels—the subarachnoid spaces. These connected but interrupted pathways are lined by a mesothelial cell-layer—the cells being of a low, somewhat cuboidal type. These cells are probably best described as being of this low cuboidal type, following previous usage, but in places they are seemingly flattened to a pavement-type. The general morphology of the cells depends apparently not only on their situation but also on their physiological state. The outer walls of these subarachnoid spaces are constituted by the inner surfaces of the arachnoid membrane, while the interrupted side-walls of the channels are formed by the arachnoidal trabeculae. And quite similarly, the inner surfaces, or floors, of these spaces are lined by the outer cell-layer of the pia mater. Thus the subarachnoid spaces may be described as being intraleptomeningeal channels, covered on the outer surfaces by the low cuboidal mesothelium of the inner surface of the arachnoid; these cells are continued inwardly along the arachnoid trabeculae to fuse directly with the cellular covering of the pia mater, which thus forms the inner boundary. The subarachnoid space (or better, spaces) furnishes, in this way, specially organized cell-lined channels for the cerebro-spinal fluid. In certain locations, particularly in the cerebello-bulbar and cerebello-pontine angles, and in the interpeduncular space, the subarachnoid pathways become enormously dilated, with a decrease in the number of trabeculae but with no alteration of the essential morphologic relations. The largest of these spaces have been called cisternae; the smaller channels have been referred to as lacunae, flumina, etc., following the terminology of Key and Retzius ('76).

The pia mater is usually described, following the French school, as one of the three meninges closely investing the central nervous system. Its relation to the subarachnoid spaces has already been described; it forms, in reality, merely a reflection of the mesothelial cells from the arachnoid trabeculae upon the nervous tissue with a minimum of supporting elements. This membrane

should not be considered as an intact cellular layer over the neuraxis, for in places it is seemingly incomplete. Thus, in the inferior velum, the pia must be perforated if an actual medial foramen of Magendie exists, and likewise, a similar condition must hold in the rhombic lateral recesses, if the two foramina of Luschka are anatomical openings. If these areas of fluid-passage are merely permeable membranes of differentiated ependyma, morphologically intact and complete, then surely the pia over the areas must likewise assume the character of a somewhat similar, permeable membrane. Again, in relation to the perivascular channels, the pia seems perforated, in a special manner, by all of the blood vessels as they enter or leave the nervous system. In these perivascular spaces, the pia apparently enters as a mesothelial lining of the outer surface of the space; a variable distance from the exterior these cells become unrecognizable and apparently are lacking, replaced by neuroglia elements. The inner walls of these perivascular spaces seem likewise covered, for a certain distance, by mesothelial cells, reflected with the vessels from the arachnoid covering of all of these vascular channels as they traverse the subarachnoid spaces. Thus, while in general the customary description of the pia mater as a membrane closely investing the cerebro-spinal axis and plunging into the cerebral sulci holds, the intact character of the pia as a cellular layer is probably incorrect. The supporting elements of the pia will not be discussed here as they have no relation to the cerebro-spinal channels.

From some aspects, the emphasis that is laid by current descriptions upon the arachnoid as a smooth membrane bridging the cerebral sulci is quite correct. Such a character, however, belongs only to the outer surface of the meninx; there the arachnoid is a complete and intact membrane, covered with low, somewhat cuboidal mesothelial cells similar to those lining the subarachnoid spaces. Between the outer and inner layers of the arachnoidea, there is a scanty supporting tissue of white fibrils and some elastic fibers. This supporting tissue likewise forms a core for the arachnoidal prolongations to the pia mater. In view of the two different characters of the arachnoidea, it

seems best to continue to use the term 'arachnoidal membrane' to refer to the outer continuous membrane and the term 'arachnoidal trabeculae' to refer to the arachnoidal prolongations from the arachnoid to the pia mater (Weed, '16 b).

Except in certain definite areas, the arachnoidal membrane on its outer surface is entirely separated from the inner surface of the dura mater. The areas of fusion represent everywhere invasions of the pachymeninx by the arachnoidea. The most frequently found of these points of fusion are the arachnoid villi,—prolongations of the arachnoid membrane into the dura so that the arachnoidal mesothelial cells come to be directly beneath the vascular endothelium of the great dural venous sinuses. These arachnoid villi have been described in adult man, in infants, and in the ordinary laboratory mammals. Histologically these prolongations are covered by the typical arachnoidal cells (low cuboidal mesothelium) usually of only a single layer but often forming whorls of many cells and presenting double-layered coverings. The core of these villi may be a strand-like network reduplicating the arachnoid spaces (as in dog) or a myxomatous ground-work simulating the perimedullary mesenchyme. In addition to the true arachnoid villi, which occur in the walls of practically all of the dural sinuses, there are found infrequently prolongations of the arachnoid into the dura in other situations. From these other prolongations and especially from the villi, columns of arachnoid cells project varying distances into the dura (Weed, ('14^b)). The arachnoid villi represent normal structures; the great enlargement of these in late adult life in human beings results in the formation of the well-known Pacchionian granulations.

The subdural space (between arachnoid and dura) is usually considered to be a part of the cerebro-spinal channels. It is a very small space, the two limiting surfaces being separated by merely a capillary layer of fluid. Whether this fluid is exactly similar to the cerebro-spinal fluid is very difficult to ascertain. Likewise our knowledge of the connections between the subdural and subarachnoid spaces is hardly definite. Hill ('96) believed the two spaces to be intimately connected, with easy

fluid-passage through foramina or by filtration. Quincke ('72), many years before Hill, found an apparent passage of granular material only in the direction from subdural to subarachnoid. It seems established, however, that certain foreign salts, in molecular solution, introduced into the spinal subarachnoid spaces do not reach the subdural space (Weed, '14 a, b). In some ways, however, the subdural space may be likened to a serous cavity.

This analogy between the subdural space and the coelomic serous cavities is supported by the definite occurrence of flattened polygonal mesothelial cells on the inner surface of the dura. The inner limiting membrane of the subdural space is not of this character as it is covered by the somewhat cuboidal mesothelium of the arachnoid. The fluid of the subdural space, in consequence, has probably a local origin from the cells lining it, for it seems anatomically well separated from the true cerebro-spinal fluid. No proof for this view has been definitely advanced. The remainder of the dura is a thick fibrous membrane, composed almost entirely of white fibrous tissue with but a negligible amount of yellow elastic tissue. It is described as having two layers in the cranium; the evidence for this rests solely upon the fact that the fibrous membrane divides to enclose the sinuses and other structures. Anatomically in the remainder of the pachymeninx there is no line of separation. In the cranium, also, the dura subserves the function of the internal periosteum of the skull, whereas in the spinal canal, it is surrounded by the fatty areolar tissue of the epidural space.

There remains to consider, under this heading, only the permeability of these membranes, in relation to the cerebro-spinal fluid. The importance of the subarachnoid spaces as fluid-channels has already been emphasized: are these spaces efficient fluid-retainers? Evidence on this phase of the problem is afforded by the spread of injection-materials of various kinds. After injections of India ink into the spinal subarachnoid spaces, the granules of carbon are entirely retained within the same spaces if the experiment be acute; after a few hours, some of the carbon may be found in the low cuboidal cells of the arachnoid. Quite similar are the results after the injection of cinnabar; in

this case also a cellular phagocytosis of the granules takes place after some hours. These suspensions of granules are, of course, abnormal in every way; the reliable test of permeability may be made only with true solutions. If an isotonic solution of potassium ferrocyanide and iron-ammonium citrate be injected into the lumbar subarachnoid spaces, the mesothelial cells enclosing the fluid-channels, are subsequently found impermeable to the foreign salts. This may be determined by precipitating the salts as prussian-blue in an acid medium and studying histologically. In such an observation, the exposed border of the cell may be covered with the precipitate but no intracellular accumulations can be found (Weed, '14 a). Hence it seems most likely that the low cuboidal mesothelium of the arachnoid meshes forms an efficient fluid-barrier; it seems impermeable to certain foreign salts and granular material except in those cases where a specific affinity may exist. This feature of impermeability of the arachnoid mesothelium does not hold in all probability for all foreign chemicals in solution, as many of these substances are undoubtedly attracted to certain cellular elements. It is impossible at present to comment on the permeability of these arachnoid cells to colloidal suspensions. But, in general, the arachnoid channels are equipped as fluid-retainers with unquestionable powers of diffusion or adsorption in regard to certain elements in the normal cerebro-spinal fluid, deriving in this way a cellular nutrition.

THE EXTRAVENTRICULAR SOURCE

In addition to the elaboration of the cerebro-spinal fluid by the chorioid plexuses, there seems fairly well established a second source of the fluid from the nervous tissue itself. The evidence for such a view of a dual source of this fluid is rather complex and dates back to His ('65 a). The earlier experiments on the production of the cerebro-spinal fluid under the influence of drugs and other chemical substances were done without taking account of a possible increase of the flow of the fluid due to an increased permeability of the cerebral capillaries; the findings from cannulae introduced into the subarach-

noid spaces are in this respect inconclusive. There is, however, considerable evidence of value for this second extraventricular source of the cerebro-spinal fluid; some of the data supporting this conception will be presented here.

As given by Mestrezat ('12) and by Mott ('10) the fluid-spaces around the blood-vessels of the central nervous system were first described by Robin in 1858. His ('65 a) by puncture-injection was able to demonstrate a complete pericellular and perivascular network in both brain and spinal cord. This network was of greater complexity and density in the gray matter than in the white. From the gray matter, His' injections passed outward in channels around the blood-vessels to spread beneath the pia in a large subpial network. Since this time, the existence of these perivascular channels has been generally accepted, although for a while they were assailed as artifacts. Whether these channels are lined by mesothelium is not definitely established; it seems most likely that the pial mesothelium runs inward with each vessel to form an outer layer of the space. The inner wall of this tube is probably similarly derived from the mesothelial covering of the vessels, which are thus protected throughout the subarachnoid spaces. This cellular covering of the perivascular tube seemingly continues inward only a short distance, neuroglia cells probably replacing on the outer surface the mesothelial elements.

The perivascular spaces connect, according to all of the best observations, with the subarachnoid spaces (His' finding of a subpial termination not being verified). Not only have many pathological findings substantiated this connection of the perivascular spaces, but physiological and toxicological evidence has aided in establishing this conception. Milian ('04), in a case of subarachnoid hemorrhage, found the perivascular system filled with blood, but the most striking demonstration of the subarachnoid connections of these spaces was made by Spina ('08, '99, '00 a, b). This worker found a punctate exudation of a clear impid fluid from the exposed surface of the brain, following great rises in systemic arterial pressure and subsequent rises in intracranial tension. Lewandowsky ('00), accepting

Spina's evidence as conclusive, hypothecated that the cerebro-spinal fluid was really the lymph of the central nervous system and was the product of the activity of the nervous tissue, poured into the subarachnoid spaces.

Mott's important observations ('10) of a great dilatation of all the perineuronal and perivascular spaces, after ligations of certain of the head arteries, offer strong anatomical evidence of the subarachnoid connections. In these cases of cerebral anemia, the dilated spaces about the nerve-cells were found to be directly connected with pericapillary spaces and these in turn were joined to the perivascular channels and the subarachnoid spaces. Mott hypothecated from these findings a drainage of the cerebro-spinal fluid from the subarachnoid spaces into the cerebral capillaries by way of the perivascular channels. The flow of the fluid, then, on such a basis would be from the subarachnoid spaces into the nervous tissue—toward the capillary bed.

In an investigation of this subject (Weed, '14 c), spinal subarachnoid injections of an isotonic solution of foreign salts were made. The salts were subsequently precipitated as insoluble prussian-blue, and hence the course taken by the solution could be verified. In the routine replacement of the normal fluid or in an injection under pressures but slightly above normal, the subarachnoid spaces, in the course of a few hours, were found to be filled with the precipitated injection-mass, but practically none of this appeared in the perivascular system. In similar injections but under pressures of about 50 mm. Hg, the perivascular spaces were sometimes filled with the foreign salts, but only to the pericapillary spaces. To secure a really complete filling of the perivascular and pericapillary spaces, a special procedure was found necessary: the subarachnoid pressure was maintained at normal by the continued injection of the isotonic foreign solution and subsequently a cerebral anemia was caused. In such a case, the experimental cerebral anemia caused a decrease in the intracranial fluid contents sufficient to cause an aspiration of the foreign solution from the subarachnoid spaces (the cranium being potentially a closed box) and to fill the perivascular system. The nerve-cells in such a case were surrounded

by a thin collection of minute prussian-blue granules; these could be traced through pericapillary and perivascular spaces to the subarachnoid spaces.

From an analysis of these results, it was concluded (Weed, '14 c) that the fluid current in the perivascular system was from nerve-cell to subarachnoid space and that by this way a small addendum of cerebro-spinal fluid drained into the meningeal spaces. This view had already on rather insufficient grounds, been advanced by Plaut, Rehm and Schottmuller ('13) and by Mes-treztat ('12). Frazier ('15) has since accepted the view of a double source of the fluid, and Halliburton ('16) inclines to a consideration of the fluid as the "lymph of the brain" but only in a restricted sense.

Such a conception of a dual source of the cerebro-spinal fluid has received support from other observations than those recorded in the foregoing paragraphs. Jacobson's important chemical studies (unpublished) have demonstrated a distinct difference between the subarachnoid fluid (product of chorioid plexuses and perivascular system) and the ventricular fluid (product of chorioid plexuses alone). The former fluid is richer in protein and poorer in sugar than the latter—a finding to be expected if the products of nerve-metabolism are poured into the subarachnoid space. Likewise, distinct serological differences between subarachnoid and ventricular fluids from the same patient have been reported. And pathologically the occurrence of intracortical cysts from dammed-up perivascular channels indicates strongly a production of fluid within the nervous tissue itself, draining outward into the subarachnoid spaces.

THE ABSORPTION OF THE FLUID

Modern anatomical evidence regarding the absorption of cerebro-spinal fluid was first brought forward by Key and Retzius ('76) in an epochal monograph. These investigators injected gelatin solutions colored with Berlin blue into the spinal subarachnoid spaces of a cadaver. The pressures used were somewhat excessive (about 60 mm. Hg), but the continuity of spinal and cranial subarachnoid spaces was demonstrated beyond

doubt. The gelatin was also found to give evidence of a direct escape of fluid from the cranial subarachnoid spaces into the great venous sinuses of the dura through the Pacchionian bodies. In addition to this major blood-vascular absorption, a lesser drainage of the fluid into lymphatic vessels was indicated; this drainage was not direct but apparently through perineural spaces around certain of the cranial nerves.

Quinke's work ('72), though published somewhat before the monograph of Key and Retzius appeared, did not antedate their earlier reports. The greater part of Quinke's data was based on the results obtained by injecting cinnabar into the spinal subarachnoid spaces and, after varying periods, studying the meninges of the animals. Quinke found these red granules rather uniformly distributed throughout the spinal region and lodged chiefly in the basilar portion of the cranium; in both regions the granules were wholly in the subarachnoid spaces. But the granules of cinnabar were held largely by phagocytic cells; this was particularly the case in the structures along the venous sinuses which he termed the Pacchionian granulations. Quinke also identified the sulphide in the cervical lymph-nodes; here again it was held in the cell-bodies of lymphocytes.

For several years after Key and Retzius, this view of the absorption of the cerebro-spinal fluid endured, but gradually with the realization that the Pacchionian granulations, as such, do not exist in infants and in the lower animals, it was felt that this view was inadequate. For the next two decades practically nothing was published on this subject; then rather suddenly considerable work of a physiological nature was done. Most of this was planned to demonstrate the venous or lymphatic drainage of the cerebro-spinal fluid. But the anatomical data included in these observations were not of great importance.

Reiner and Schnitzler ('94, '95) injected salt solutions containing potassium ferrocyanide into the spinal subarachnoid spaces and recovered the ferrocyanide from the jugular vein in from thirty to forty seconds after the injection—physiological proof of a rapid blood-vascular absorption. Similarly, though with a somewhat slowed stream, olive oil was recovered from the

jugular vein. These investigators state that, as Pacchionian granulations do not occur in the animals used, other pathways of absorption for the cerebro-spinal fluid must exist.

Following Reiner and Schnitzler, a number of workers offered physiological evidence of the pathways of absorption of cerebro-spinal fluid. Leonard Hill ('96) was able to trace saline solution colored with methylene blue "straight into the venous sinuses" after spinal injection. While the abdominal viscera were colored with the blue within a few minutes, the cervical lymphatics were not colored until after one hour. Hill found also that serum apparently passed into the sinuses as readily as did saline. Ziegler ('96) observed that, after introduction of potassium ferrocyanide into the cerebro-spinal fluid, the substance could be identified in ten seconds in the vena facialis posterior and only after thirty minutes in the cervical lymph channels. Similarly Lewandowsky ('00) identified sodium ferrocyanide in the urine of animals within thirty minutes after intraspinal injection.

Although Spina's ('98, '99, '00) experiments may be adversely criticized because of the employment of excessive pressure, they still offer collateral evidence of great value in regard to the drainage of cerebro-spinal fluid. This worker introduced fuchsin solutions into the subarachnoid spaces of animals, but recently killed or under anesthesia, and ascertained that the venous absorption was by far the more important. He further found that with increasing pressures of injection the lymphatic channels function the more readily and carry away a great proportion of the fluid.

This idea of a greater venous absorption of the fluid was also brought forward by Cushing ('02 a, b), in the course of a study of the effects of local and general cerebral compression. After subdural rupture of a mercury-filled rubber bag, used to secure local compression, Cushing found the mercury globules in the great dural sinuses, in the diploetic vessels, in the jugular veins and in the right heart, but none was present in the cervical lymphatic chain. Cushing's observations of a direct passage of mercury, from the spinal subarachnoid space of a cadaver, directly into the superior sagittal sinus are quite analogous.

With intraspinous injection of non-absorbable gases, the same venous channels could be made out, the gaseous bubbles being apparent in the jugular veins, but not in the cervical lymphatics. From these experiments, Cushing hypothecated other channels than the Pacchionian granulations as the functionally active pathway of escape for the cerebro-spinal fluid, and suggested a valve-like mechanism of fluid-passage between the subarachnoid spaces and the great dural sinuses. Recently ('14) he has adopted a different view.

Several other views have been advanced by workers in this field. Mott ('10), from a study of the brains of monkeys in which an experimental anemia had been caused, suggested that the cerebro-spinal fluid was absorbed in the cerebral capillaries by way of the perivascular system. Assuming that this fluid was similar to other body-fluids, Mott conceived that by diffusion and osmosis the blood in the cerebral capillaries would receive water and carbon dioxide from the cerebro-spinal fluid in the pericapillary spaces and in return the blood would give salts and sugar to the cerebro-spinal fluid. Mott's chemical observations recorded a high content in the cerebro-spinal fluid of carbon-dioxide—a finding which would seemingly suggest that the fluid had received already these products of metabolism. Dandy and Blackfan ('13) likewise consider the drainage of cerebro-spinal fluid "a diffuse process from the entire subarachnoid space," basing their conclusions upon the results of subarachnoid injections of phenolsulphonaphthalein. Their results with this solution with intact cerebro-spinal spaces coincide with those given above for the absorption of true solutions; with granular material (india ink) there was found practically no absorption from these channels—a finding which coincides with the results obtained by Quineke.

The idea of an absorption of cerebro-spinal fluid solely by way of lymphatic vessels has been developed by Cathelin ('12) in a recent monograph. He has rather sweepingly condemned all of the preceding work indicating a major venous drainage, defending on insufficient grounds his contention of a sole lymphatic drainage by way of the perivascular and perineural sheaths.

Goldmann ('13) likewise inclines somewhat to the idea of lymphatic absorption, basing his conception on the staining of cervical lymph-nodes after intraspinous injection of trypan blue. While acknowledging the limitations of his method and the weight of evidence in favor of venous absorption, Goldmann points out that much of the earlier work is valueless because of failures to control pressures.

With this somewhat contradictory work as a basis, an investigation was undertaken (Weed, '14 a, b, c) to determine if possible the actual pathways by which the cerebro-spinal fluid was returned to the general blood-stream. It was felt that most of the observations already made were not conclusive as demonstrating anatomical pathways, for the morphological studies reported had all been made after injections of viscous colloidal fluids (gelatin) or of suspensions of granular material (cinnabar, india ink). The physiological findings after injections of true solutions (methylene blue, potassium or sodium ferrocyanide, phenolsulphonaphthalein, fuchsin, etc.) offered much more reliable evidence of a major venous absorption, but no anatomical pathways had been demonstrated. After critical analysis of the methods used and the criteria necessary for reliable results, true solutions, isotonic with the body-fluids were injected, under pressures but slightly above the normal, into the spinal subarachnoid spaces of anesthetized animals. A solution of foreign salts, potassium ferrocyanide and iron-ammonium citrate, in a 1 per cent concentration was used; the course of this solution could subsequently be histologically traced if the tissue was fixed in toto in an acid medium, precipitating prussian-blue. In addition to these injections at low-pressures in the anesthetized animals, simple replacements of the normal cerebro-spinal fluid by the foreign solutions, without altering the normal tension, were made. These injections under low pressures were continued usually for several hours to secure complete filling of the subarachnoid channels; after a replacement, the animals were kept alive for about the same length of time. The ferrocyanide-citrate solution proved of great value as it was not attracted to any specific cell-elements and as it was non-toxic

within the subarachnoid spaces. In this way, a physiological method of ascertaining an anatomical pathway was employed; alterations in the method were made to investigate certain phases of the problem.

From this work (Weed, '14 a, b, c) certain apparently definite results were brought forth. Accepting as evidence of the normal pathway the course taken by the solution (as determined by the resultant precipitate of prussian-blue) in the procedures approaching the physiological, it was found that the solution rather rapidly spread through the subarachnoid spaces of the spinal cord. In the cranial cavity, the injection first passed into the basilar regions (in agreement with Quinke and Goldmann) and thence slowly filled the subarachnoid spaces over the cerebral hemispheres. There was no penetration anywhere of the mesothelial cells enclosing the subarachnoid spaces, showing them to be impenetrable to this foreign true solution—an important feature in the reliance placed on the results obtained by this method. The fluid was absorbed directly into the venous sinuses by way of the arachnoid villi, which have already been described in a foregoing section of this communication. In these structures, the precipitated granules could be traced directly from the cerebral subarachnoid spaces; they were accumulated in the delicate core of the villi. The foreign solution (as evidenced by the granules) had left the villi by passing through the mesothelial cells of the arachnoid and also through the endothelial cells of the venous sinuses, thus demonstrating a direct absorption into the dural sinuses. The passage into the venous stream seemed largely a matter of filtration from a point of higher pressure (subarachnoid space) to a point of lower pressure (venous sinus), although the factors of diffusion and osmosis were not absolutely ruled out. In this connection the observations of Wegefarrth ('14 b) and also of Dixon and Halliburton ('14) regarding cerebro-spinal pressures are of interest. It was found also that the absorption of true solutions from the cranial subarachnoid spaces was a much more efficient and rapid process than was the corresponding absorption from the spinal region. Suspensions of particulate matter, however, were invariably

found not to pass into the venous sinuses but to lodge largely in the meshes of the subarachnoid spaces and in part in the arachnoid villi.

In addition to this major venous absorption through arachnoid villi directly into the great dural sinuses, an accessory drainage by way of the lymphatic system was described, the observations being based on the same method of experimentation (Weed, '14 b). This is a much slower, less efficient means of escape for the cerebro-spinal fluid, normally caring for but a small fraction of the total drainage in all probability. This lymphatic absorption is wholly indirect; the fluid reaches the true lymphatic vessel only outside of the dura and of the cranium. The mechanism for this drainage is by way of perineural spaces around the spinal nerves to a slight extent but chiefly around certain of the cranial nerves—particularly the olfactory branches. The precipitated prussian-blue (evidence of the distribution of the foreign true solution) could be made out in a perineural arrangement around the olfactory branches in the nasal membrane; the granules escaped into the interstitial tissue and from this the absorption into the lymphatic vessels took place. On the course of certain of the cranial nerves (optic, acoustic, etc.) such an indirect, secondary lymphatic drainage apparently does not occur.

Almost simultaneously with this publication, Frazier and Peet ('14) reported the results of subarachnoid injections of methylene blue, isamine blue, trypan red and blue, and phenolsulphonaphthalein. The trypan blue did not reach the lymph-glands of the neck until after several hours, but the phenolsulphonaphthalein was detected in the torcular blood in from two to three minutes. They regarded the blood-vascular absorption as most important and the lymphatic drainage as a minor accessory mechanism.

Since these more recent observations were issued, Dixon and Halliburton ('16), in the course of their excellent studies on cerebro-spinal fluid, have reported the results of their physiological experiments on the absorption of the fluid. They found, in confirmation of the results given above, no absorption of particulate matter and a free and rapid absorption of true

solutions. Between the two types, they discovered a much slower absorption of colloidal solutions, the larger molecules being absorbed more slowly than the smaller. This report represents a very valuable contribution to the subject of absorption. Furthermore, Dixon and Halliburton agreed that the spinal portion of the subarachnoid spaces did not possess great powers of absorption as compared with the cranial drainage, which was much more efficient. This observation, in accord with previous work, rendered the hypothesis of Dandy and Blackfan ('13) untenable. Likewise it casts perhaps doubt on the possibility of Mott's theory ('10) being correct, for it is difficult to assume a power of absorption of cerebro-spinal fluid by the cerebral capillaries without granting a similar function to the spinal. For other reasons, however, these theories of drainage seem insufficient (Weed, '14).

Dixon and Halliburton, moreover, could not find, during the period of observation, evidence of any lymphatic absorption by tapping the thoracic duct; the whole absorption seemingly went by way of the blood-vascular system. They conclude, as does also Halliburton ('16) that "the fluid probably reaches the venous sinuses by way of the microscopic arachnoid villi." Their findings in regard to the lymphatic absorption are probably to be accounted for by the very slow process of drainage along this pathway.

Thus it seems fair to assume, from the evidence presented above, that the absorption of cerebro-spinal fluid is a dual process, being chiefly a rapid drainage into the great dural venous sinuses, and in small part, a slow escape into the true lymphatic vessels, by way of an abundant but indirect perineural course.

EMBRYOLOGY OF THE CEREBRO-SPINAL SPACES

With this conception of the processes of the cerebro-spinal fluid apparently indicated by the investigations up to the present, the embryology of the pathways for this fluid will be discussed. At the Christmas meeting of the American Association of Anatomists in 1915, the results of replacing, in living pig-embryos, the existing ventricular fluid by a foreign true solution

were presented (Weed, '16 a). The method of replacement provided for the exchange of fluids without increasing the intraventricular pressure, and the embryos were subsequently kept alive for an hour or so, the foreign solution being non-toxic and not attracted to specific cellular elements. In this work, an isotonic solution of potassium ferrocyanide and iron-ammonium citrate was used; these salts were precipitated subsequently as prussian-blue and the resultant granules were considered to represent the course of the normal cerebro-spinal fluid.

By this method of investigation, it was found that in pig-embryos up to about 14 mm. in length, the replaced true solution remained wholly within the central canal of the spinal cord and within the cerebral ventricles. In the smaller stages (about 8 mm.) the injection showed no peculiarities but in a somewhat larger stage (13 mm.) a definite, very dense oval of precipitated prussian-blue could be made out in the central portion of the roof of the fourth ventricle. It was through this oval in the rhombic roof that the fluid passed from the cerebral ventricles into the extraventricular tissues, in the stages in pig-embryos of over 14 mm. This extraventricular spread of the fluid indicated that the balance between the intraventricular production of fluid and the increasing volume of the cerebral ventricles was destroyed; it was of considerable interest that this overthrowing of the balance should occur coincident with the development of tuftings in the chorioid plexuses of the fourth ventricle. From this stage of the initial outpouring of fluid from the cerebral ventricles, the further spread was not extensive until a length of about 18 mm. was reached in the pig-embryo. At this older stage, two areas, one in each half of the now divided rhombic roof, affording means of fluid-passage from the ventricles, were made out. From both of these areas the fluid escaped into the perimedullary tissues in exactly the future subarachnoid arrangement. In pig-embryos of over 18 mm., the further extension of the pathways of the embryonic cerebro-spinal fluid occurred rapidly. At 19 mm., the peribulbar tissues had become filled with the fluid; and within a couple of millimeter's growth, the whole perispinal spaces were invaded by the fluid, the de-

velopment apparently being from above downward. At the same time, the basilar mesencephalic region was found filled by the fluid and an infundibular extension very quickly occurred. At a stage of about 26 mm., a complete perimedullary distribution of the replaced fluid was recorded—the arrangement of fluid at this period being that of the adult.

This escape of fluid from the roof of the fourth ventricle into the extraventricular tissues occurs through two areas of ependymal differentiation (Weed, '16 a) The superior of these areas makes its appearance as a definite oval in the roof of the fourth ventricle (cf. replacement injection of stage of 13 mm.) and represents an earlier differentiation of the original ependymal lining of this ventricular cavity. Quite similarly, as this oval comes to lie in the superior half of the rhombic roof after this structure is transected by the laterally developing chorioid plexuses, a second area of differentiation of the ependyma develops in the inferior half of the roof. The superior area reaches its maximal anatomic differentiation and apparent functional significance at a stage in the pig of 19 to 20 mm.; from this point on, it undergoes a regression, sacrificed to the greater development of the chorioid plexuses and the downward growth of the cerebellum. The inferior area, however, persists as a functional area of fluid-passage, gradually occupying the whole inferior velum chorioidea. Whether a permanent persistence of this area during adult life occurs, cannot be answered until more data regarding the foramen of Magendie are at hand. The differentiation of the ependyma in these areas is largely a transformation to a flattened, elongated type of cell. Through the cell-cytoplasm in this area, as through a cellular membrane, fluid passes in accordance with the laws of filtration and possibly of diffusion (Weed, '17).

A year after the work detailed above was presented, Keegan ('17) reported in an abstract, the results of a similar investigation, carried out independently. Keegan made use of the same isotonic solution of potassium ferrocyanide and iron-ammonium citrate, and also of a solution of the citrate alone. In the main, Keegan's results were similar to those given above, particularly

in regard to the occurrence of two areas of ependymal differentiation in the roof of the fourth ventricle. Instead of making use of replacements, he employed a finely drawn glass cannula and made injections directly into the lateral ventricle of living embryos. Such a method, because of the unavoidable increase in the intraventricular pressure, hardly seems to offer results of as great reliability as are afforded by the replacement method. Keegan reports that the injection of the double solution in living rabbit embryos did not undergo any extraventricular spread until a stage of 17 days was reached, although the specimens showed an oval aggregation of granules in the rhombic roof; solutions of the iron-ammonium citrate alone, however, gave early extensions through the roof of the ventricle. Similar results were obtained in chick-embryos, the roof being impermeable to the double solution and permeable to the citrate alone. It seems rather difficult to understand this relative impermeability of the rhombic roof to the combined solutions of the citrate and ferrocyanide; this combination has been used with success by several investigators during the last few years, in many problems of absorption through membranes. The reactions of the rabbits and chicks may be such as to prevent such fluid-passage. After injection with the citrate solution, Keegan records an extraventricular spread shortly before the chorioid plexuses develop; this is somewhat at variance with the results in pig-embryos after replacements (Weed, '16 a).

The cerebro-spinal fluid of the embryo, passing from the cerebral ventricles, is poured into the perimedullary mesenchyme (Weed, '16 b). This mesenchyme about the central nervous system in the early stages (about 10 mm. in the pig) is a loose tissue characterized by a somewhat small mesh, with frequent oval nuclei; the cytoplasm is largely prolonged into the syncytial formation. As soon as the embryonic ventricular fluid enters this tissue, a differentiation is begun. This changing process begins in the peribulbar tissues and spreads thence both downward about the spinal cord (here the process is almost synchronous with the peribulbar) and more slowly upward about the cerebral hemispheres. This differentiation consists of a modification of the primary small-meshed mesenchyme into

the larger arachnoid spaces. Thus, with the initial outpouring of fluid, a process of dilatation of the perimedullary mesenchyme begins; the spaces between the syncytial strands seem first to become enlarged and filled with a more albuminous fluid (as evidenced by the resultant coagula). Subsequently with further differentiation, some of the cytoplasmic strands are broken off; the processes recede and the whole cell-body may become merely an integral part of a persisting, much strengthened strand—a future arachnoidal trabecula. In certain areas, as in those of the future cisternae, the process of breaking down of the mesenchymal mesh and the formation of new arachnoid spaces reaches its maximum, for here the spaces become relatively very large and the number of trabeculae correspondingly fewer.

Associated with this differentiation of the perimedullary mesh into the larger arachnoid spaces, there occurs at the same time a process of mesenchymal condensation. Such a thickening becomes evident not only in the development of the persisting arachnoidal trabeculae, but chiefly in the formation of the outer continuous arachnoid membrane. This first appears as a thin zone of secondary condensation, between the enlarging spaces of the perimedullary mesenchyme and the thickening blastema of the bony coverings. In this way, the perimedullary mesenchyme is subdivided into two zones as described by His ('65 b) and Kölliker ('79). Farrar ('06) has also recorded in a brief note a somewhat similar division of the mesenchyme in the chick. However, this zone of condensation represents not only the arachnoid membrane (as distinguished from trabeculae) but also the inner surface of the dura. In this narrow thickening of the mesenchyme, differentiation occurs, permitting a cleavage of the membranes (50 mm. in the pig). Thus, in the formation of the arachnoid there occur two processes—a dilating, enlarging process forming the subarachnoid spaces, and a condensing, thickening process resulting in the formation of the arachnoidal trabeculae and membrane.

While the mesenchymal dilatation in the perimedullary spaces is proceeding, the pia mater is being formed out of the same mesenchymal elements. At first, with the extensive capillary

plexus investing the nervous system, the cells of the mesenchyme form apparently a single and very often a double layer about the neuraxis, in an adult pial relation. The breaking down of the original small mesenchymal mesh still leaves a layer of pial cells closely attached to the nervous tissue; these are continuous with the cells covering the arachnoid trabeculae. Some of these cells also must be considered as invading the nervous tissue to some extent to form lining elements for the perivascular spaces. This embryonic pia is a comparatively efficient membrane, resisting forces from within (as shown by the occurrence of subpial extravasations after experimental rupture of the nervous system from excessive injection pressures) and being impermeable to solutions from without (as in the subarachnoid extensions of replaced foreign solutions).

The mesenchymal cells, enclosing the newly formed subarachnoid spaces and also covering on its outer surface the arachnoid membrane, undergo a rather gradual differentiation into the low cuboidal mesothelial elements which in adult life cover these fluid-spaces. A few of the mesothelial cells, both in the trabeculae and in the membrane, are devoted finally to the formation of the meager supporting tissues of these structures. The differentiation of pial cells is wholly similar to the process in the so-called arachnoidea, for this pial membrane both embryologically and functionally is merely the inner retaining layer of the subarachnoid channels.

After the appearance of the secondary condensation of mesenchyme, separating the perimedullary tissue into two zones, the formation of the dura may be traced. In the outer zone, extending to the blastemal condensations, a distinct increase in the number of mesenchymal elements may be made out; all of this outer zone of tissue is ultimately devoted to the formation of the fibrous portion of the dura. The process begins in the basilar regions of the cranium and spreads upward, following somewhat the differentiation of the arachnoid spaces and of the developing cranium. Fibrous tissue may soon be detected in this denser zone and the gradual formation of a dense fibrous membrane may be made out. The inner surface of the dura,

however, develops in connection with the outer surface of the arachnoid membrane; these together are included in the secondary zone of condensation. As soon as a distinct separation of the two membranes over the cerebral hemispheres can be made out in fetal pigs (50 mm.), a polygonal mesothelial cell-pattern on the inner surface of the dura may be demonstrated by silver reductions.

A combined study of the processes of dilatation of the mesenchymal spaces and of specimens prepared after replacements of the embryonic ventricular fluid by the ferrocyanide solutions, shows that the mesenchymal cells are impermeable to this true solution, that both the pial cells and the secondary condensation present barriers to the spread of the fluid, and that the course taken by the replaced fluid coincides with the thinning of the arachnoid mesh and with the presence of an increased amount of the albuminous coagula (suggesting the presence of the protein-rich embryonic fluid). Thus the anatomical limits of the spread of the foreign solution coincide apparently with the physiological use of these fluid-channels.

A full account of the embryology of the cerebro-spinal spaces is being published as one of the Contributions to Embryology of the Carnegie Institution of Washington (Weed, '17). In the account given above, only the more general processes have been presented; for the evidence and more complete information, reference to the Carnegie publication must be had.

DISCUSSION

The processes, then, of the cerebro-spinal fluid are in many respects fairly well established. The evidence for the current beliefs is extremely good for certain viewpoints concerning the anatomical pathways involved and for certain physiological phenomena. When, however, the viewpoint here expressed is compared to the accounts appearing in the current text-books and in some of the current literature, a marked diversity of opinion is brought forth.

It does, however, seem established, on definite and firm grounds, that the cerebro-spinal fluid is largely produced within

the cerebral ventricles by specially differentiated structures, the chorioid plexuses. After traversing the ventricles, this fluid passes from the fourth ventricle into the subarachnoid spaces; these are channels adapted for fluid-passage and permit the distribution of the fluid everywhere about the central nervous system. From these spaces, the fluid returns to the blood-vascular system, the mode of absorption being a process largely of filtration and of diffusion through arachnoid villi into the dural sinuses.

But in addition to the production of the cerebro-spinal fluid by the chorioid plexuses, there is apparently a further addition of fluid by way of the perivascular system. This is evidently derived primarily from the capillaries of the central nervous system; it passes from these structures directly into the pericapillary spaces and thence to each nerve-cell or outward through the perivascular channels into the subarachnoid spaces. The amount of fluid derived by this method is undoubtedly small; it is presumably somewhat different in its chemistry from the fluid elaborated by the chorioid plexuses. There is, likewise, an accessory mechanism for the absorption of fluid, in addition to the major drainage into the venous sinuses of the dura. This minor method of escape for the cerebro-spinal fluid is by way of the perineural spaces (particularly those of certain of the cranial nerves) indirectly into the lymphatic system. This is, according to the best evidence, a slow mechanism; it probably cares for but a very small portion of the total absorption of fluid.

It might well be conceived that much of the confusion regarding the relations of the cerebro-spinal spaces to the lymphatic system has had its origin in such a conception of an accessory method of production of cerebro-spinal fluid and in this indirect absorption by the lymphatics. This view, however, does not include, wholly, the real explanation of the difficulty about the lymphatic relationship; the idea of lymph channels in the dura and in the leptomeninges was advanced many years before the problems of production and absorption of the cerebro-spinal fluid were really modernized.

This older view that the meninges themselves possessed lymph vessels was presumably based chiefly on the work of Arnold ('38) who described three definite layers of lymphatic vessels in the pia-arachnoid. This work has been quite widely quoted, due to His' ('65, a) somewhat comparable findings about thirty years afterward. His was able to make out a definite subpial plexus of supposed vessels after intramedullary puncture-injection—a method which today would hardly be relied upon for such an investigation. In the dura, Böhm ('69), by similar puncture-injection with Berlin blue, demonstrated an apparent spread of the foreign mass into definite channels resembling the network of other parts of the body.

The evidence given by these investigators probably represents the best offered for the definite occurrence of lymphatic vessels within the meninges. It was brought out before the lymphatic system was definitely found to be a series of closed tubes and before the more modern criteria of lymphatics were established. Puncture-injections should yield a definite plan of lymphatic network, substantiated by histological evidence of an endothelial channel. But in the leptomeninges, puncture-injections or extensions from other injections, show a peculiar network—not lymphatic but representing merely the filling and distension of tissue-channels in these membranes. In the pachymeninx, likewise, puncture-injections outline a definite network, resembling only superficially a lymphatic plexus. This was reported by Böhm ('69) using Berlin blue and was also observed in several cases on human dura after india ink injections (Weed, '14c). But the channels in the dura, injected by this method, are not lymphatic vessels; they are not lined by endothelium nor is the pattern of the network typical in any way of a lymphatic plexus. These dural channels are merely unlined spaces between the dense strands of fibrous tissue; similar unlined spaces may be demonstrated by similar injection in any dense fibrous membrane.

But in the dura there is another factor which contributes to the possible confusion. This is the occurrence in that membrane of definite columns of cells, differentiated clearly by their

morphology and tinctorial reactions from the dural tissue. These cell-columns, often simulating to some extent a vascular endothelial tube, could be confused with lymphatics, but all of the evidence demonstrates that these cell-columns are arachnoidal in origin, representing intradural prolongations of the arachnoid cells and forming a potential channel for cerebro-spinal fluid. They are primary pathways related to the veins and are not secondary or lymphatic.

The more recent work, making use of the more definite criteria for lymphatic vessels and excluding rigorously tissue-spaces or other potential channels, has not established the occurrence of definite endothelial-lined lymphatic vessels in the three meninges. Such a view receives great support from the work of Sabin ('12) who has never observed, in many injections of the head region in embryos, any extension of lymphatic vessels into the dura. Likewise, all recent anatomical work on these perimedullary membranes in adults has failed to demonstrate any lymphatic channels. Hence it seems essential at the present time to conclude that there occur, in the meninges, no true endothelial-lined lymphatic vessels. It is, however, obvious that evidence of such a negative character is more likely of future contradiction than analogous positive demonstrations. Every argument, though, points to an avoidance of the meninges by the developing lymphatic sprouts.

If then, there is a definite lack of lymphatic vessels in the meninges, there must necessarily be a similar lack of these vessels in the nervous tissue itself. This view is very definitely established in every way; the true endothelial-lined lymphatic vessels do not occur in the cerebro-spinal axis. With a lack of these vessels in the meninges it would be very difficult to assume that any could occur in the central nervous system. In this connection, it must be emphasized that the subpial plexus of His has not been confirmed; all the evidence is against the acceptance of such a conception. In the place, possibly, of the true lymphatic vessels, there occur in the nervous tissue the perivascular spaces. These partially lined channels have in the past been termed the 'perivascular lymphatic spaces' or

'perivascular lymphatics' but in more recent publications, the 'lymphatic' has been omitted, so that the spaces seem best termed 'perivascular' spaces. Streeter ('17) has very recently modified likewise the terminology of the spaces about the ear, terming the perilymphatic spaces the 'periotic.'

These perivascular channels, according to the data presented in a foregoing section of this paper, pour into the subarachnoid spaces a small amount of fluid. It represents probably the medium of fluid-exchange between the blood-capillary and the nerve-cell, the excess of this intramedullary fluid being eliminated by the perivascular system. It subserves, then, in a way, the function of the tissue-fluid of other regions and tissues of the body, being analogous to the other tissue-fluids but lacking the possibility of direct absorption by the lymphatic system. This intramedullary fluid is, in this restricted sense, an 'extra-vascular lymph' or, as Halliburton terms it ('16), "the lymph of the brain." But such a conception of the function and distribution of this intramedullary tissue-fluid in no way justifies the application of the word 'lymph' to the cerebro-spinal fluid. For it does seem essentially important to continue to regard the fluid existing within the true lymphatic vessels as being the only 'lymph.' Tissue-fluids (or extravascular lymph, a poor term) in most of the other parts of the body have a definite and close physiological relation to the lymphatic vessels; here in the central nervous system, the tissue-fluid from the nerve-cells represents merely a small addition to the cerebro-spinal fluid already traversing the subarachnoid spaces. Thus, as no true endothelial-lined lymphatic vessels apparently exist within the meninges or central nervous system, the cerebro-spinal fluid cannot be regarded as 'lymph.'

In many other respects, the processes of the cerebro-spinal fluid exclude it from the designation of 'lymph.' The anatomical characteristics of its pathway—at first, with an ependymal lining and subsequently, with a low, cuboidal mesothelium—surely separate this system from the lymphatic vessels. The embryological formation of the cerebro-spinal spaces is quite different from the origin and development of the lymphatic

system (cf. Sabin '13 a, b). For here in these intramembranous channels, there occurs a dilatation and breaking down of the original mesenchymal tissue-spaces to form the final subarachnoid spaces. This is a process entirely apart from our present conceptions of a centrifugal growth of a lymphatic system from a venous endothelial origin. In these perimedullary spaces, the developmental arrangement has no primary vascular relations; it follows apparently the physiological use of these spaces as fluid-channels. This mode of formation of the cerebral-spinal spaces is quite similar to the process which results in the formation of the anterior chamber of the eye. In this aqueous reservoir, however, the destruction of the mesenchymal syncytial strands becomes complete (except in the spaces of Fontana), a phenomenon which has its direct analogy in the formation of the subarachnoid cisternae.

The analogies between the processes of the cerebro-spinal fluid and of the aqueous humor are very marked and very numerous (Henderson ('10), Wegefarth and Weed, ('14)). The fluid in both eye and brain is chemically identical; it is in both places produced by similar epithelial structures—the ciliary processes and the choroid plexuses. The fluid first traverses epidermal-lined structures—posterior chamber and cerebral ventricle—passing through foramina into the mesodermal spaces (anterior chamber and subarachnoid spaces). The major absorption in both cases is into venous sinuses (canal of Schlemm and great dura! sinuses) through the mechanism of cellular prolongations, the pectinate villi in the eye (Wegefarth, '14a) and the arachnoid villi (Weed, '14b). The accessory production of fluid is likewise probably of the same character in the two organs.

Thus in many ways, the cerebro-spinal fluid must be looked upon as being a characteristic body-fluid, quite apart from the ordinary tissue-fluids and from the true lymph of the endothelial-lined vessels. It may be considered identical with the aqueous humor (and possibly with the fluid of the periotic spaces) and may be likened, as Halliburton ('16) has suggested, to a physiological salt solution. In only a very restricted and minor sense may it possibly be referred to as lymph; may not this usage of the term be definitely discarded?

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